Pattern Recognition Receptors

Seeing and responding to danger

Tom Monie

Lecture overview

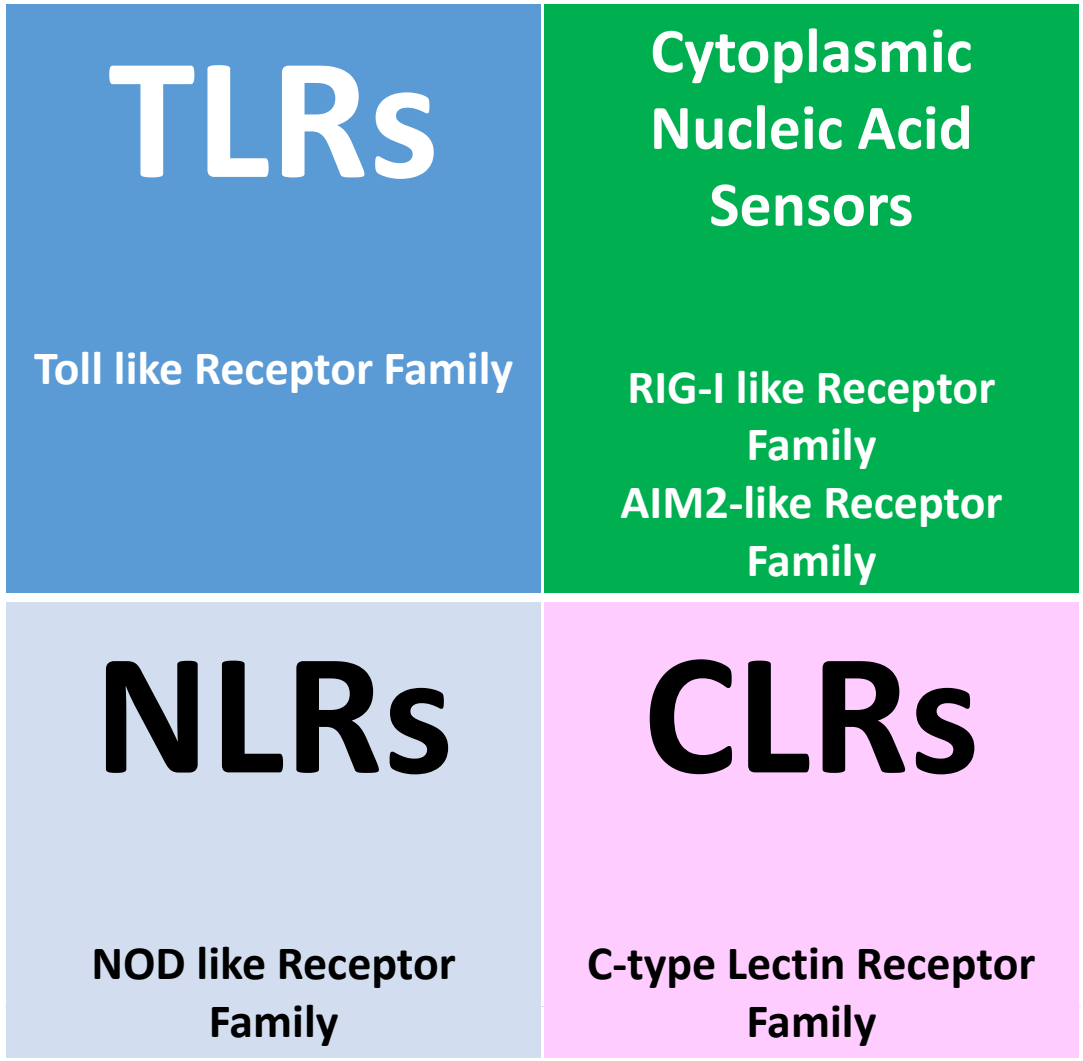
- PRR signalling pathways
  - Activation and signal transduction
- Ligand recognition by TLRs and NLRs
- Macromolecular signalling complexes
  - Myddosome and inflammasome
- Detection of LPS
  - cell surface versus cytoplasm
- PRRs and disease

What is a PRR?

- A protein receptor that recognises molecules that indicate a potential danger to the cell.
- PRR activation leads to the initiation of a protective immune response
- PRR ligands can be derived from exogenous and endogenous sources

PRR families

- **Pattern Recognition Receptors (PRRs) act as sentinels for the detection of cellular danger.**



PRRs also include proteins such as scavenger receptors, CD11b and CD14

PRR ligands: primarily PAMPs, but an increasing number of DAMPs

- Bacterial cell wall components (lipopolysaccharide (LPS), bacterial lipoproteins, lipoteichoic acid (LTA), peptidoglycan, bacterial DNA other bacterial associated proteins)
- Viral constituents- primarily viral DNA and RNA
- Fungal cell wall components eg zymosan, fungal hyphae
- Some constituents of protozoa
- Helminth and other parasitic constituents??
- Apoptotic mammalian cells
- Putative endogenous ligands eg HSP60 (DAMPs)
- Auto-antigens?

Receptor	Microbial product
TLR-1 (with TLR-2)	Mycobacterial lipoprotein Triacylated lipoproteins
TLR-2 (with TLR-1 or TLR-6)	Gram-positive bacteria Peptidoglycan, lipoteichoic acid Zymosan, liparabinomannan Bacterial glycolipids, yeast mannan GPI anchors of *Trypanosoma cruzi* LPS from *Leptospira interrogans* LPS from *Porphyromonas gingivalis* (more cylindrical)
TLR-3	Viral dsRNA, synthetic polyinosinic acid: cytidylic acid (poly I: C)
TLR-4	Gram-negative bacteria LPS (conical shape), pneumolysin Lipid A (strictly cylindrical, antagonist) LPS from *Rhodobacter sphaeroides* (strictly cylindrical) Flavolipin from *Flavobacterium meningosepticum* Respiratory syncytial virus protein F *Aspergillus fumigatus* hyphae HSP 60 and 70, hyaluronan Fibronectin A domain, fibrinogen Necrotic cells, saturated fatty acids, taxol (only in mice)
TLR-5	Flagellin
TLR-6 (with TLR-2)	Mycoplasma lipoproteins, lipoteichoic acid, peptidoglycan
TLR-7 and TLR-8	Single-stranded RNA, imidazoquinalones
TLR-9	CpG DNA, hemozoin
TLR-10	Unknown
TLR-11	Uropathogenic bacteria Profilin-like protein molecule in *Toxoplasma gondii*
TLR-12	Profilin-like protein molecule in *Toxoplasma gondii*
RIG-1	5′ triphosphorylated dsRNA
MDA-5	Long dsRNA
Protein kinase R	dsRNA
Dectin-I	β-Glucans
Mannose receptor	Liparabinomannan
f-MLP receptor	f-MLP
Moesin	LPS

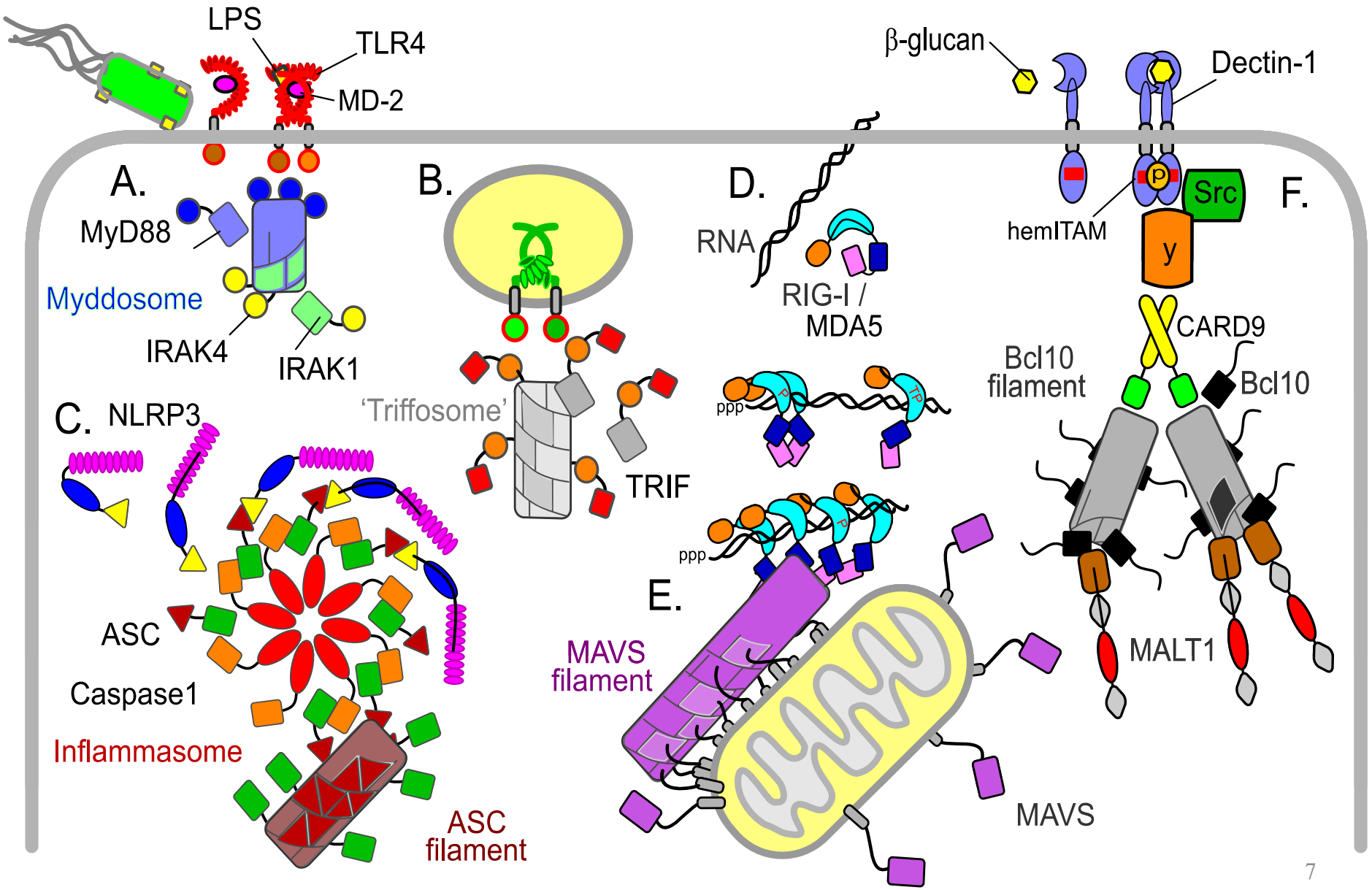
A few example PRR ligands

PRR ligands are generally highly conserved molecular patterns that provide a key function.

This means that they are less prone to mutation and variation.

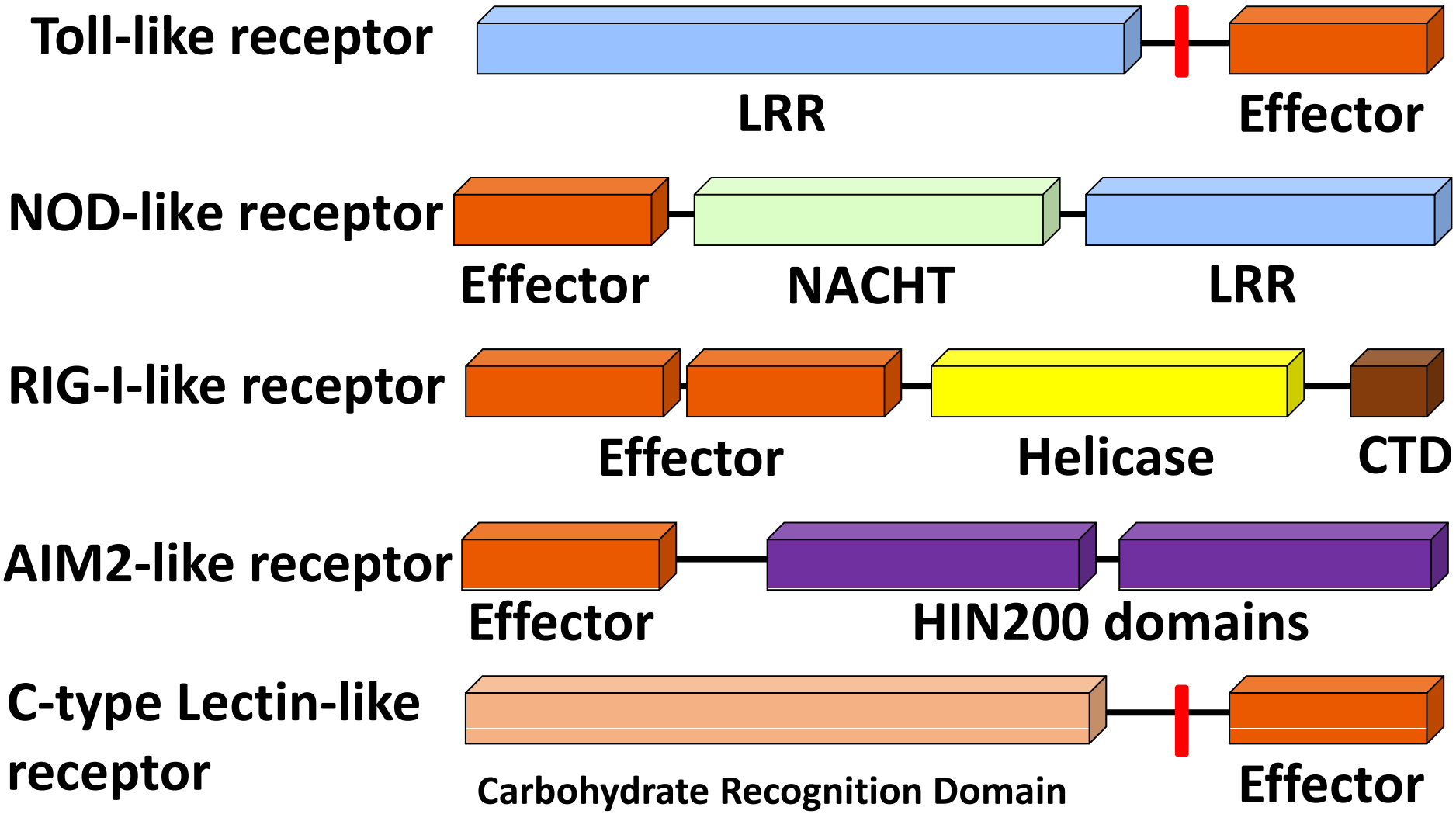
Chassaing et al Biomedical Journal 2014 **DOI**:10.4103/2319-4170.130922

PRR localisation



Bryant et al *Pharmacological Reviews April 2015 vol. 67 no. 2 462-504* doi: 10.1124/pr.114.009928

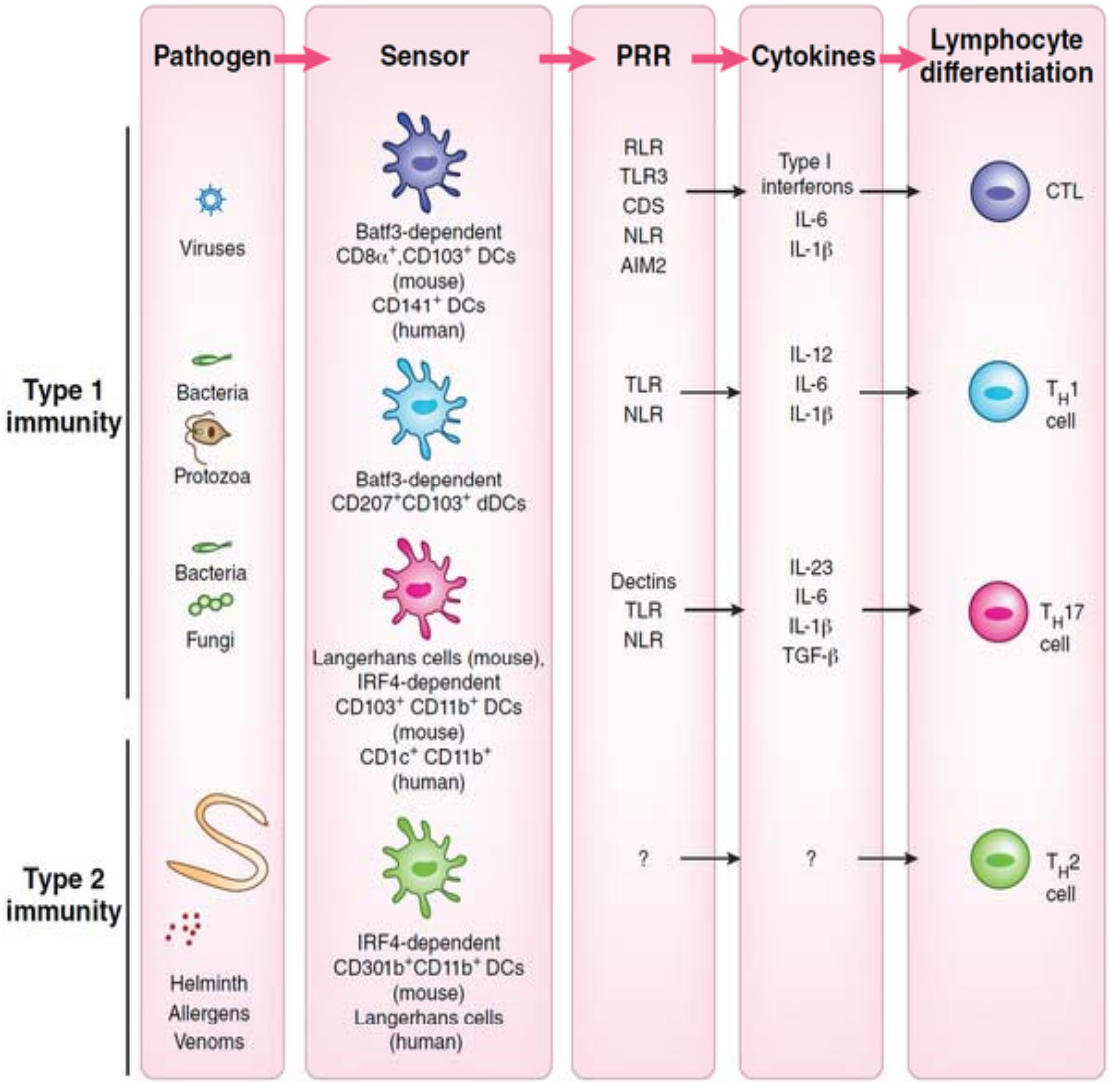
PRR Domain Structure



Consequences of PRR activation

- Ligand recognition switches on PRR signalling pathways to activate innate and adaptive immunity.
- At its most simple PRR activation activates the host innate immune response to induce localised and specific cytokine production to control pathogens and remove danger.
- PRR activation results in an immune response finely balanced between protection and destruction
- Over, prolonged or inappropriate activation of PRRs can result in disease or even death
  - Sepsis, type II diabetes, cryopyrin-associated periodic syndromes

PRRs and adaptive immunity



- It is becoming increasingly clear that PRR activation has a key role in determining the precise nature of the adaptive immune response

Iwasaki and Medzhitov Nature Immunology 16, 343–353 (2015) doi:10.1038/ni.3123

PRRs and vaccines

Table 1. Triggering of the Innate and Adaptive Components of the Immune System by Major Adjuvants

Adjuvant	Major Immunostimulatory Component(s)	Innate Receptors or Pathway Activated	Principal Immune Responses Stimulated
Licensed Adjuvants			
Alum	aluminum salts	NLRP3 inflammasome (?)	Ab, Th2 (+ Th1 in humans)
MF59 and AS03	squalene-in-water emulsions	tissue inflammation (no receptors defined)	Ab, Th1 + Th2
AS04	MPL plus alum	TLR4 and inflammasome (?)	Ab, Th1
Adjuvants in Widespread Experimental Use or in Late Stage Clinical Development			
Poly-IC (also Poly-ICLC)	synthetic derivatives of dsRNA	TLR3, MDA5	Ab, Th1, $CD8^+$ T cells
MPL and formulations (AS01, AS02)	MPL and QS-21	TLR4 (MPL), ? (QS21)	Ab, Th1
Flagellin, flagellin-Ag fusion proteins	Flagellin from *S. typhimurium*	TLR5	Ab, Th1 + Th2
Imiquimods	imidazoquinoline derivatives	TLR7, TLR8 or both	Ab, Th1, $CD8^+$ T cells (when conjugated)
CpG oligodeoxynuceotides and formulations (IC31, QB10)	synthetic phophorothioate-linked DNA oligonucleotides with optimized CpG motifs	TLR9	Ab, Th1, $CD8^+$ T cells (when conjugated)
CAF01	trehalose dimycolate (cord factor)	Mincle	Ab, Th1, Th17
ISCOMS and ISCOMATRIX	saponins	mechanism undefined	Ab, Th1+ Th2, $CD8^+$ T cells
IFA (and Montanide formulations)	mineral or paraffin oil + surfactant	mechanism undefined	Ab, Th1 + Th2
CFA	IFA + peptidoglycan, trehalose dimycolate	NLR, inflammasome, Mincle, TLR?	Ab, Th1, Th17

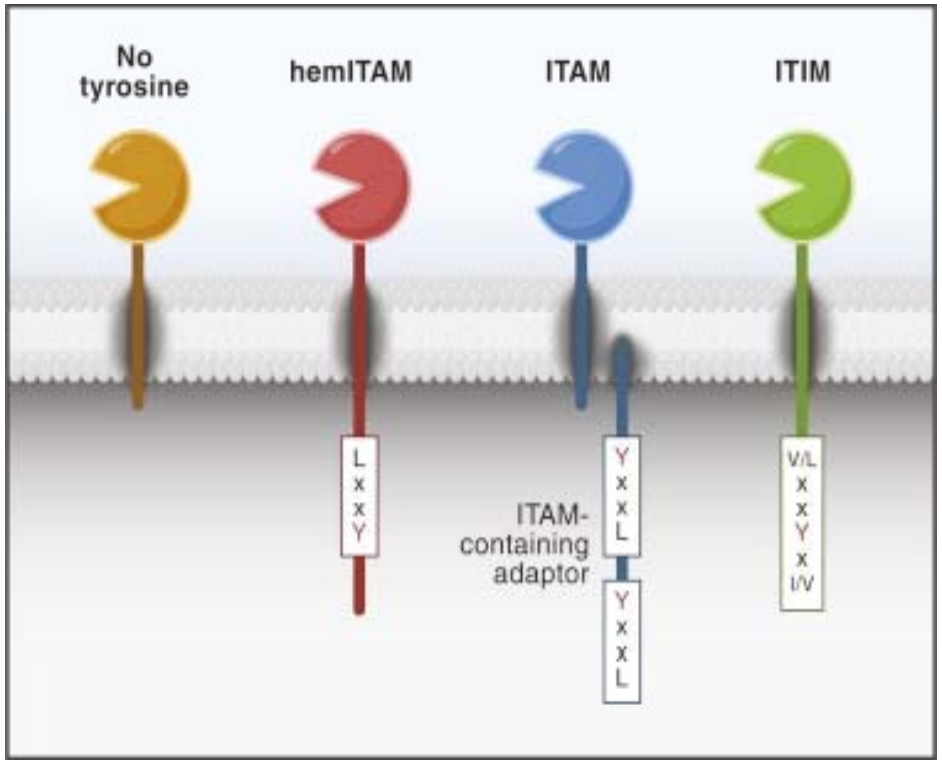
The principal immune response stimulated is based on results from human and mouse studies, although it may be limited to one species in some cases. Where indicated, conjugation of TLR ligand to antigen is necessary to obtain significant $CD8^+$ T cell responses.

Coffman *et al* Immunity. 2010 Oct 29; 33(4): 492–503.

C-type Lectin Receptor (CLR) signalling

- C-type lectin Ca^{2+}-dependent carbohydrate-binding lectins.
- CLRs share at least one carbohydrate recognition domain with conserved residue motifs: determines carbohydrate specificity of the CLR.
- Can have one or more domains that are homologous to carbohydrate recognition domains but do not always bind carbohydrate structures.
- CLRs exist both as soluble and transmembrane proteins (PRRs).

C-type Lectin Receptors (CLRs)



•Classified based on type of Tyrosine-Based Signaling Motif, which determines potential to engage pathways that induce or modulate gene expression rather than signal for endocytosis.

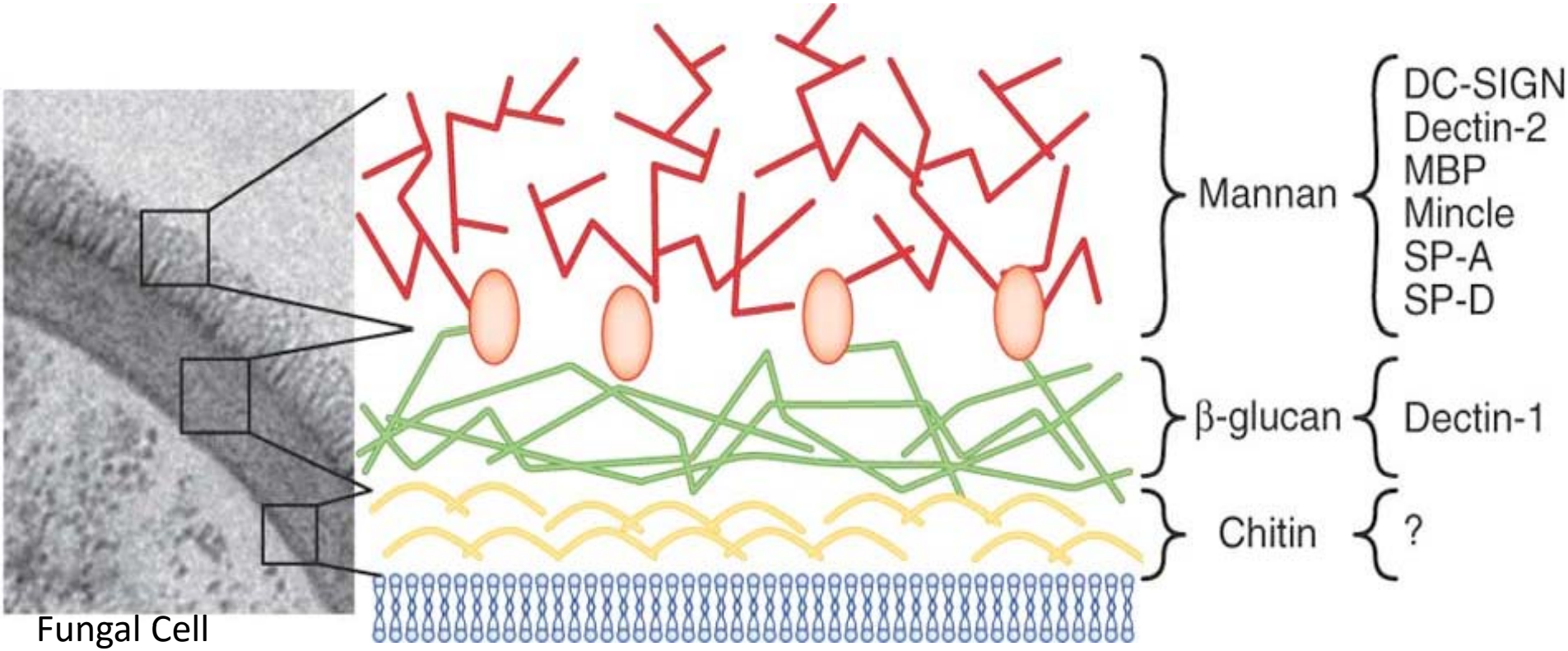
•CLRs bearing a hemITAM or ITIM motif are all type II transmembrane proteins; hence the motif is written with the tyrosine depicted as membrane distal.

Examples:

1. *mannose receptor, CD206, CLEC13D: virus, bacteria, fungi, protozoa*
2. *langerin, CD207, CLEC4K: virus, bacteria but primarily fungi*
3. *DEC-205, CD205, CLEC13B: virus, bacteria, dead cells*
4. *Dectin-1, CLEC7A: mycobacteria, fungi*

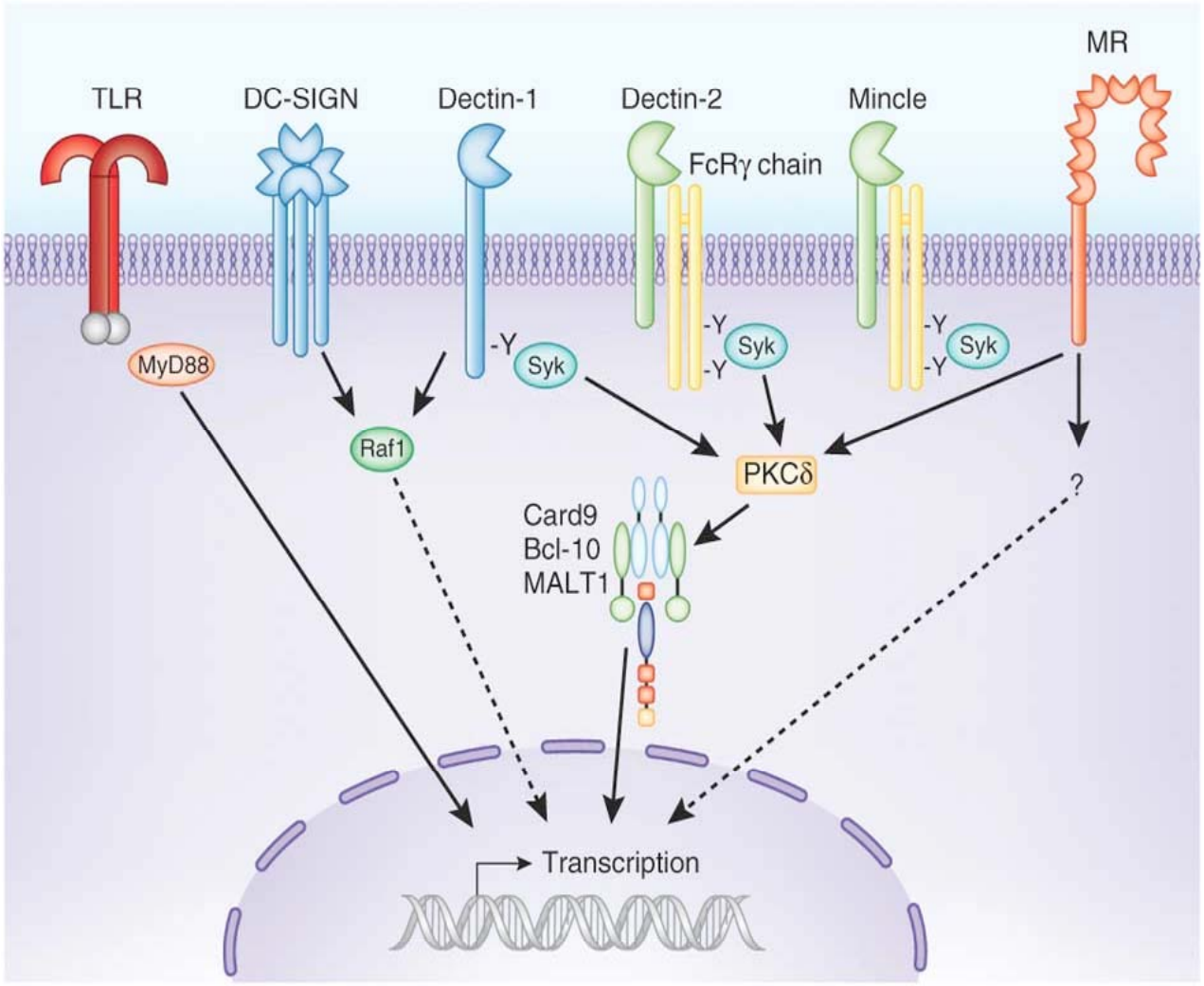
Myeloid C-type Lectin Receptors in Pathogen Recognition and Host Defense Immunity 2011 34 (5) 651–664 Fabiola Osorio , Caetano Reis e Sousa

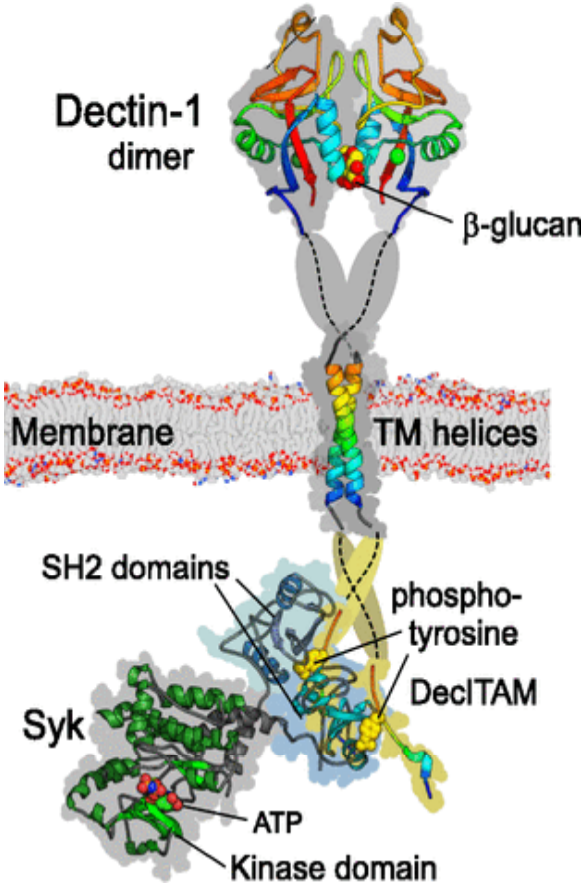
Fungal recognition by CLRs



- CLRs recognise pathogens through mannose, fucose and glucan carbohydrate structures (mannose - viruses, fungi and mycobacteria; fucose - some bacteria and helminths; glucan - mycobacteria and fungi)
- CLR activation leads to internalization of pathogen, its degradation and subsequent antigen presentation

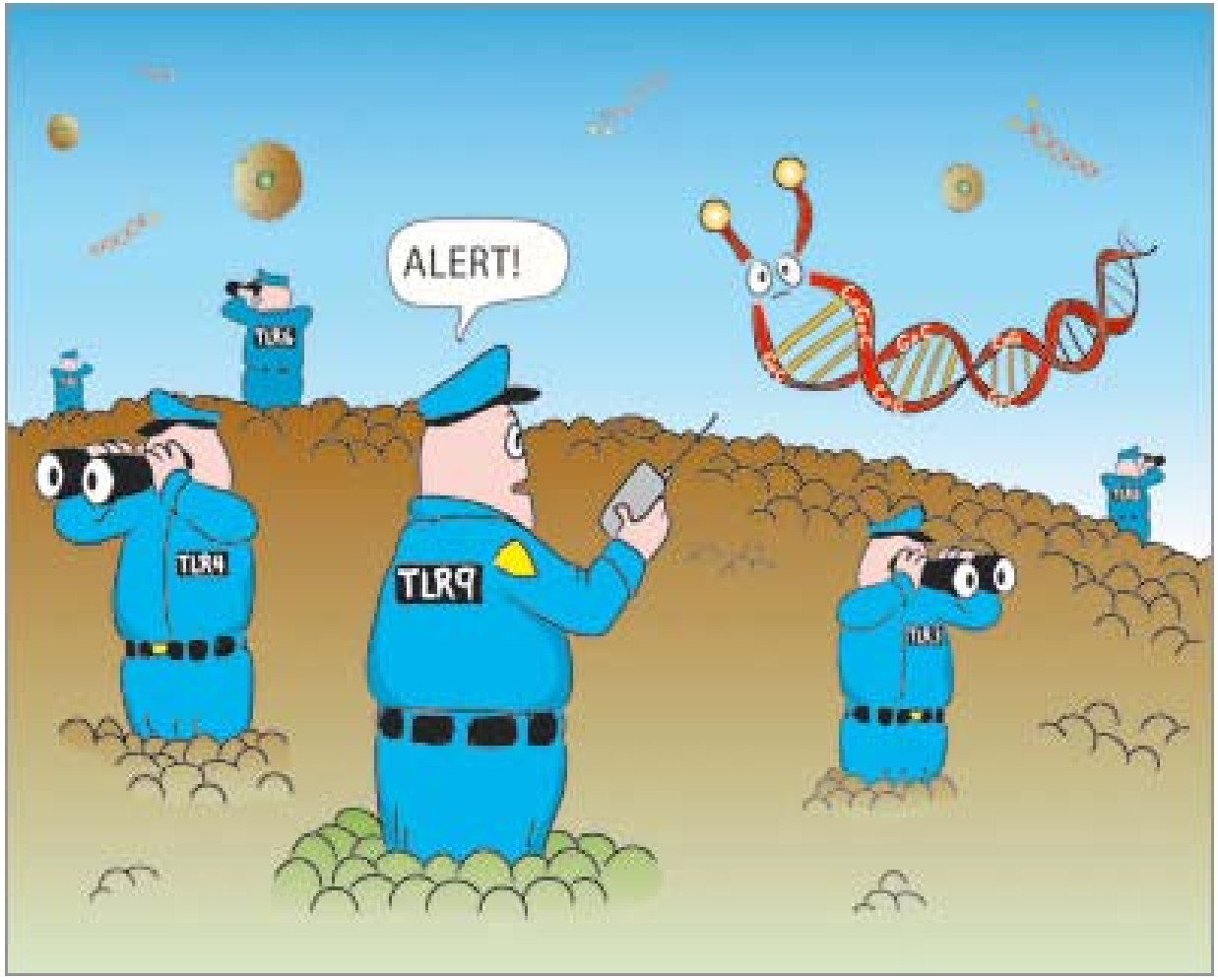
Fungal Receptor Signalling





Bryant et al *Pharmacological Reviews April 2015 vol. 67 no. 2 462-504* doi:
10.1124/pr.114.009928

Dirty Little Secrets: Toll and Toll-like receptors: a lot has happened since 1989

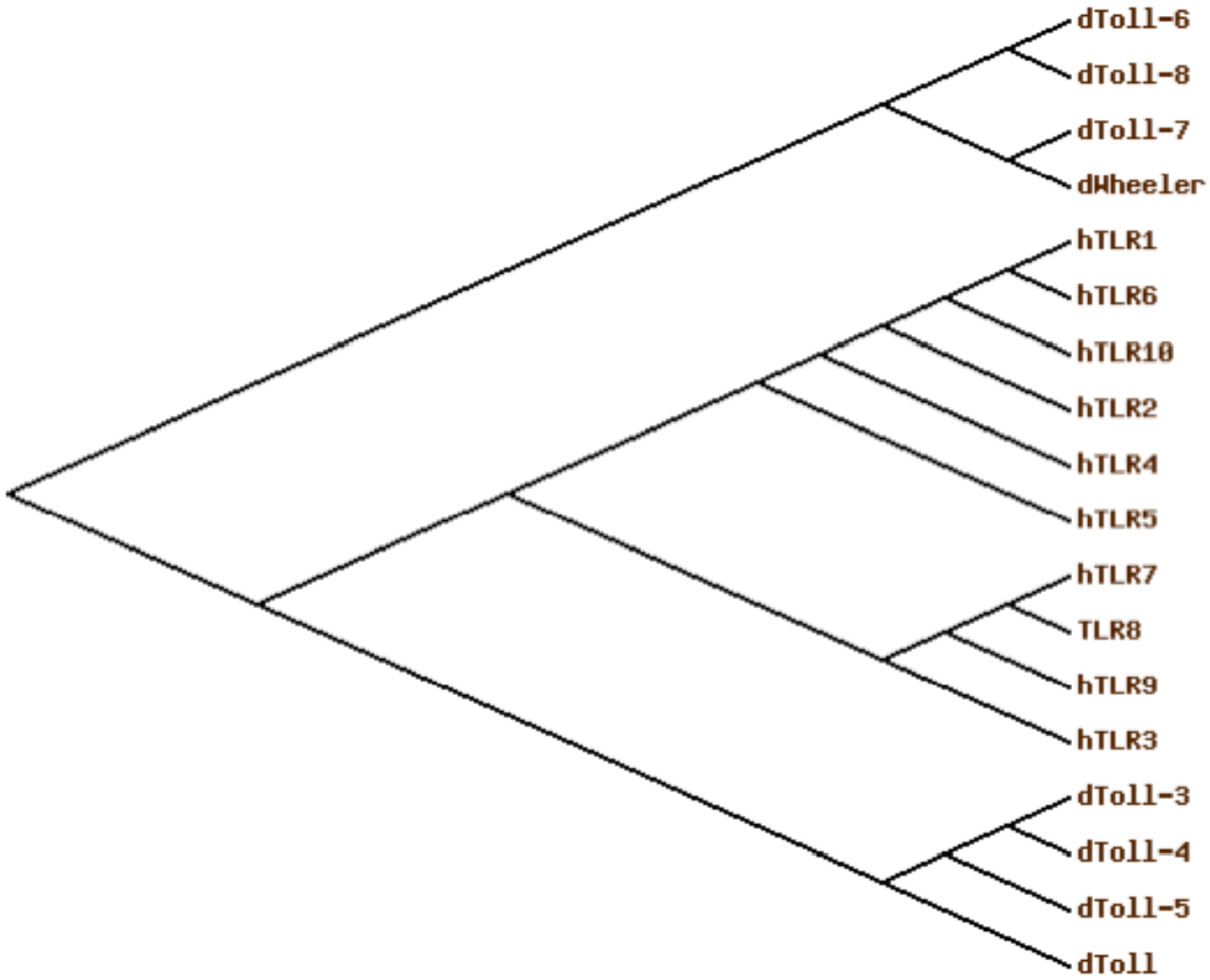


Including a Nobel Prize awarded in 2011 to Jules Hoffman and Bruce Beutler

Lessons to be learnt from flies: The drosophila Toll receptor family

- First identified in *Drosophila* in genetic screen for mutants affecting embryonic pattern formation. Toll = mad/amazing (German)
- One of about 12 genes involved in dorso-ventral pattern formation.

Phylogenetic tree of the Tolls.

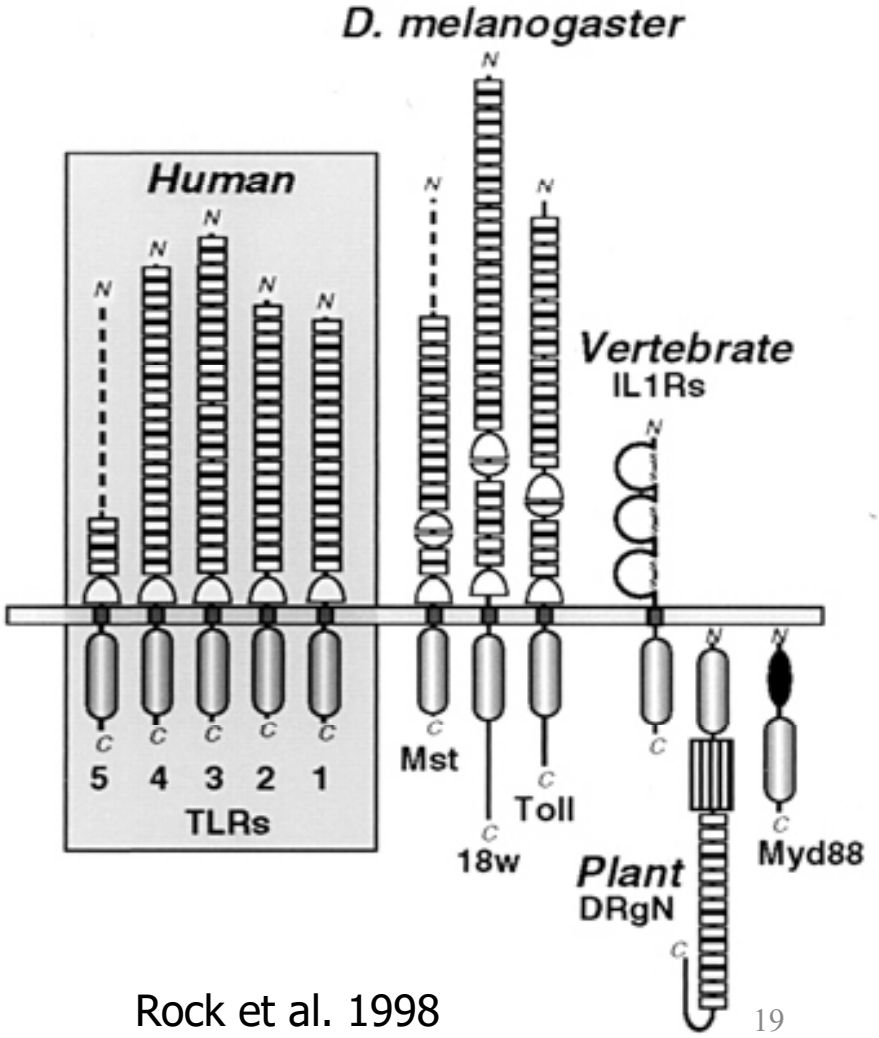


Toll and Toll-like receptors (TLRs)

Architecture of TLRs

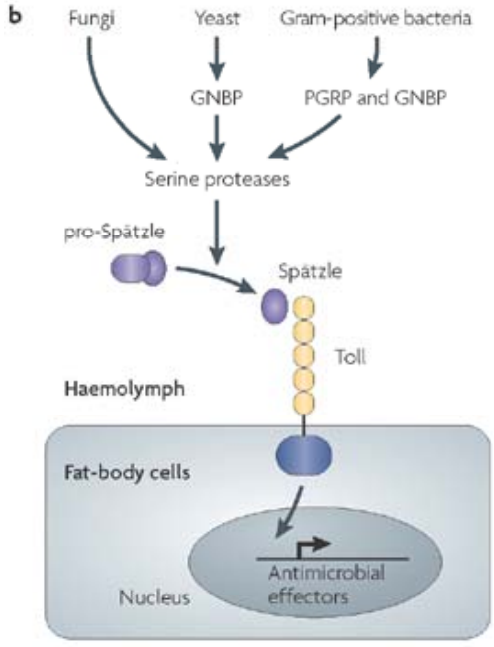
- The extracellular region of TLRs contains blocks of leucine-rich repeats surrounded with cysteine-rich regions.

- The intracellular signalling domain (TIR homology domain) is shared by TLRs, Interleukin-1 receptor, plant resistance R proteins and adapter proteins (MyD88, Mal/TIRAP, Tram and Trif).

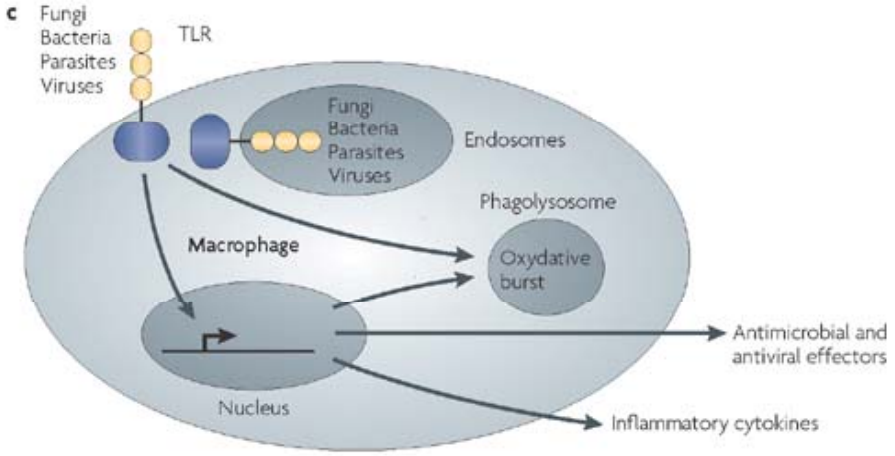


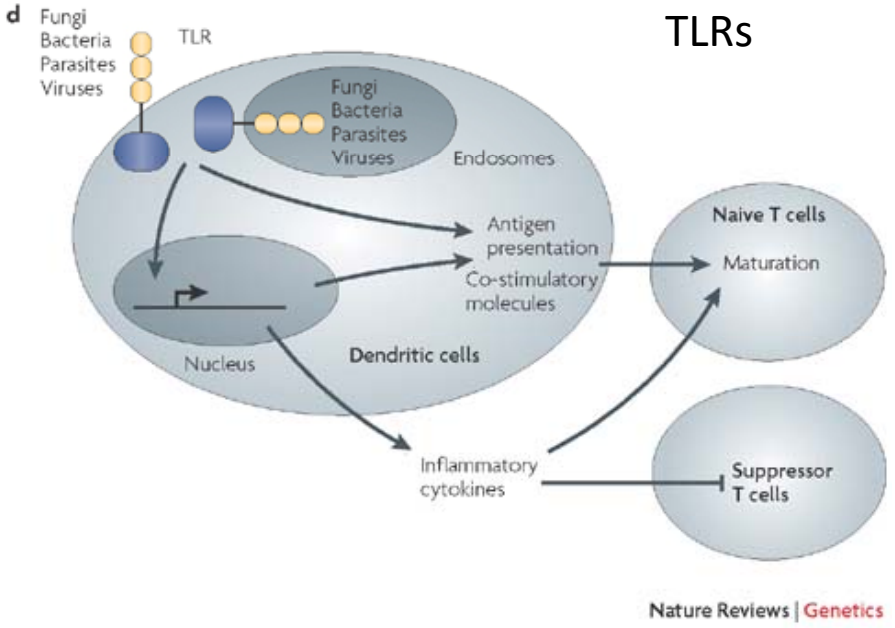
Rock et al. 1998

Similarities and differences in signalling



Toll

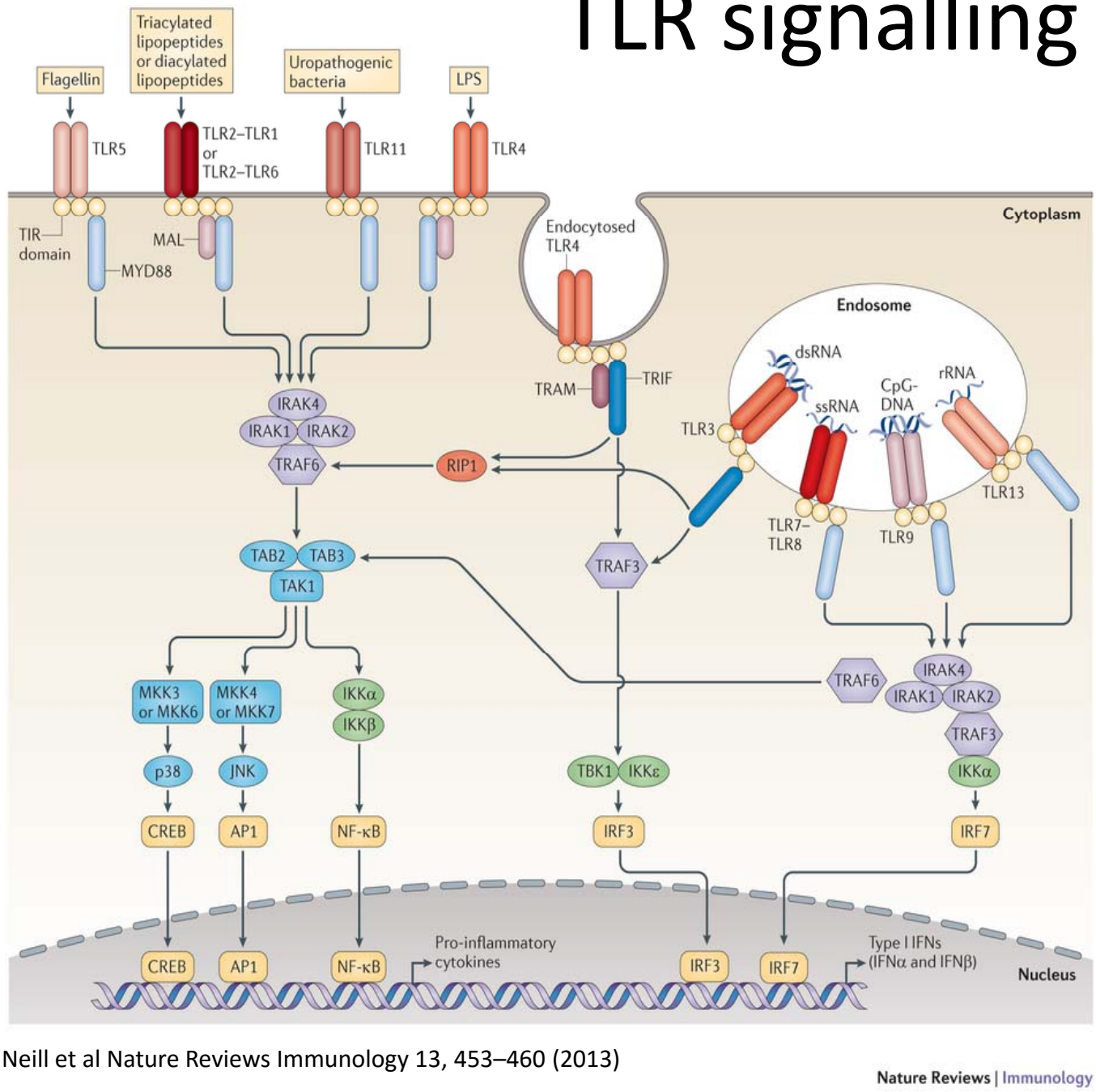




TLRs

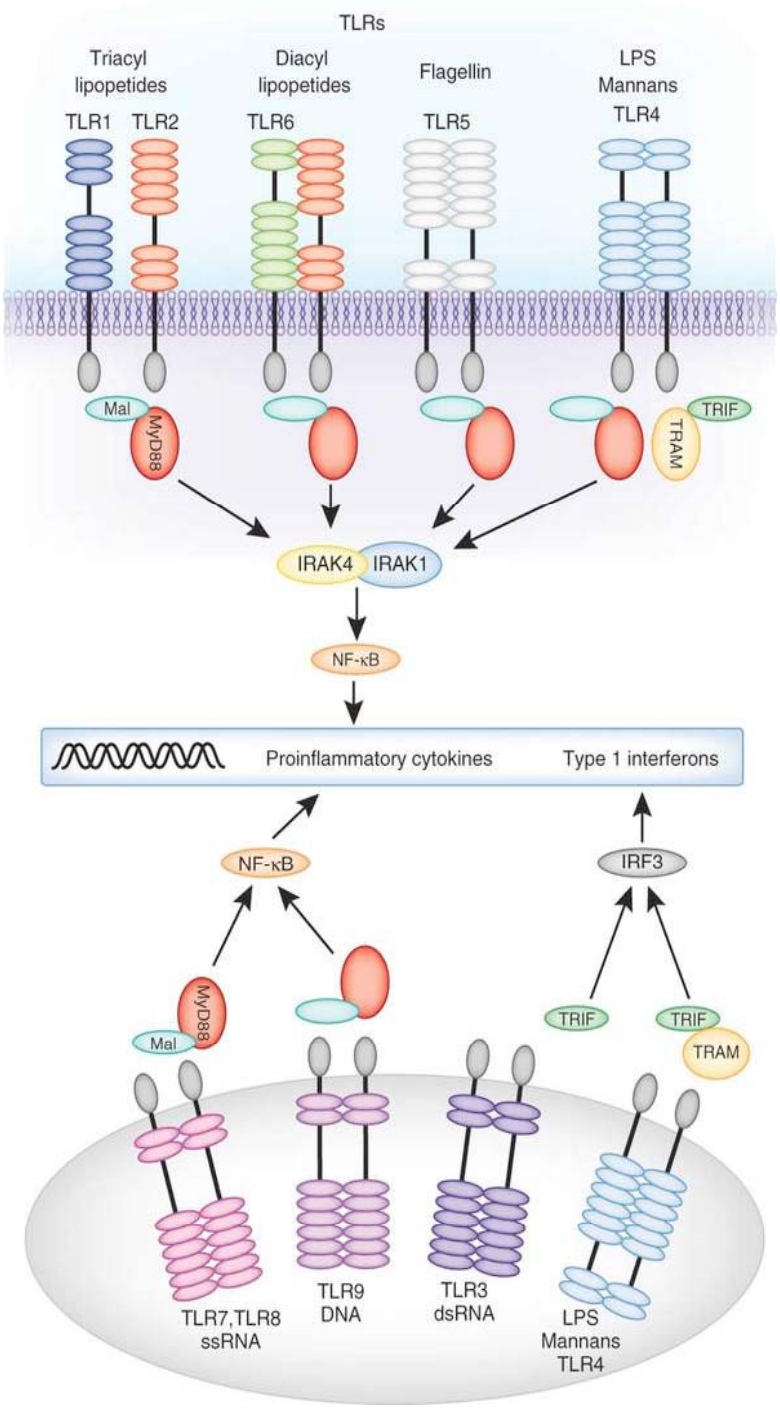
Nature Reviews | Genetics

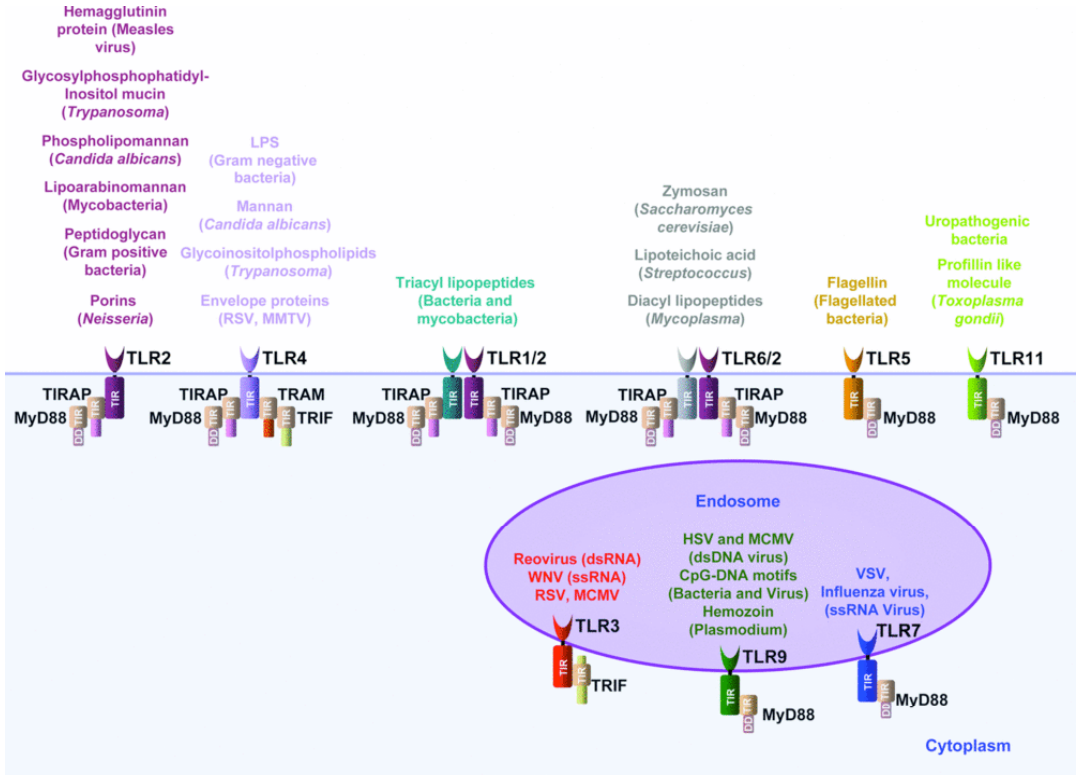
TLR signalling pathways



O'Neill et al Nature Reviews Immunology 13, 453–460 (2013)

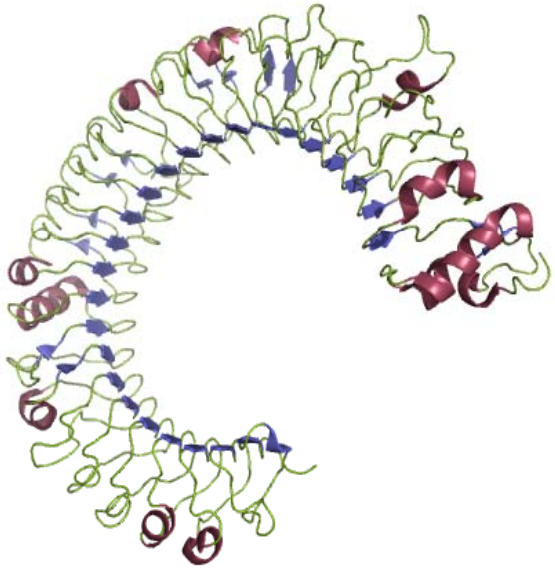
TLRs and their ligands





Ligand specific TLR activation

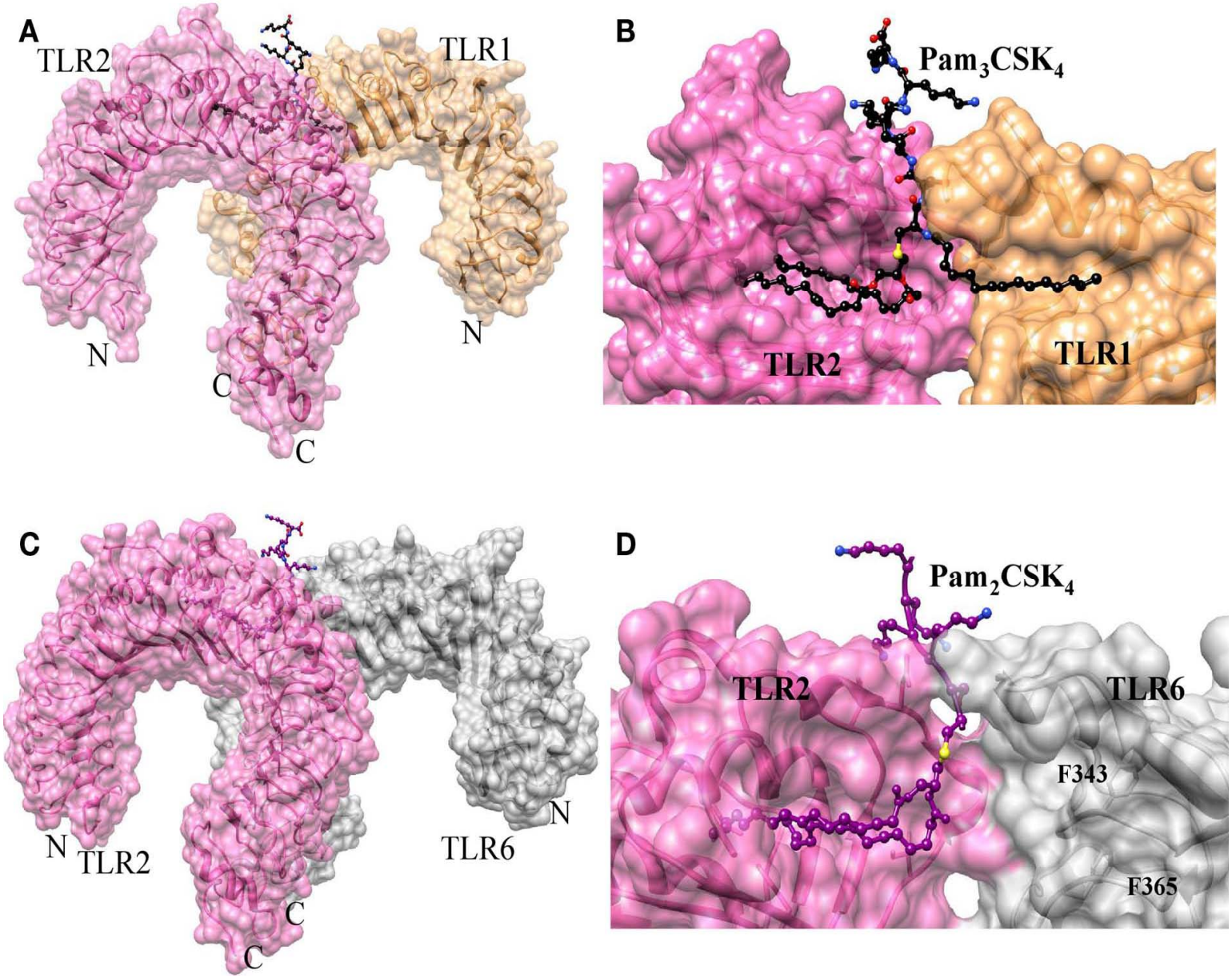
- TLR activation requires direct contact between the ligand and the LRR domain resulting in conformational change in the TIR domain and signal propagation.



LRR

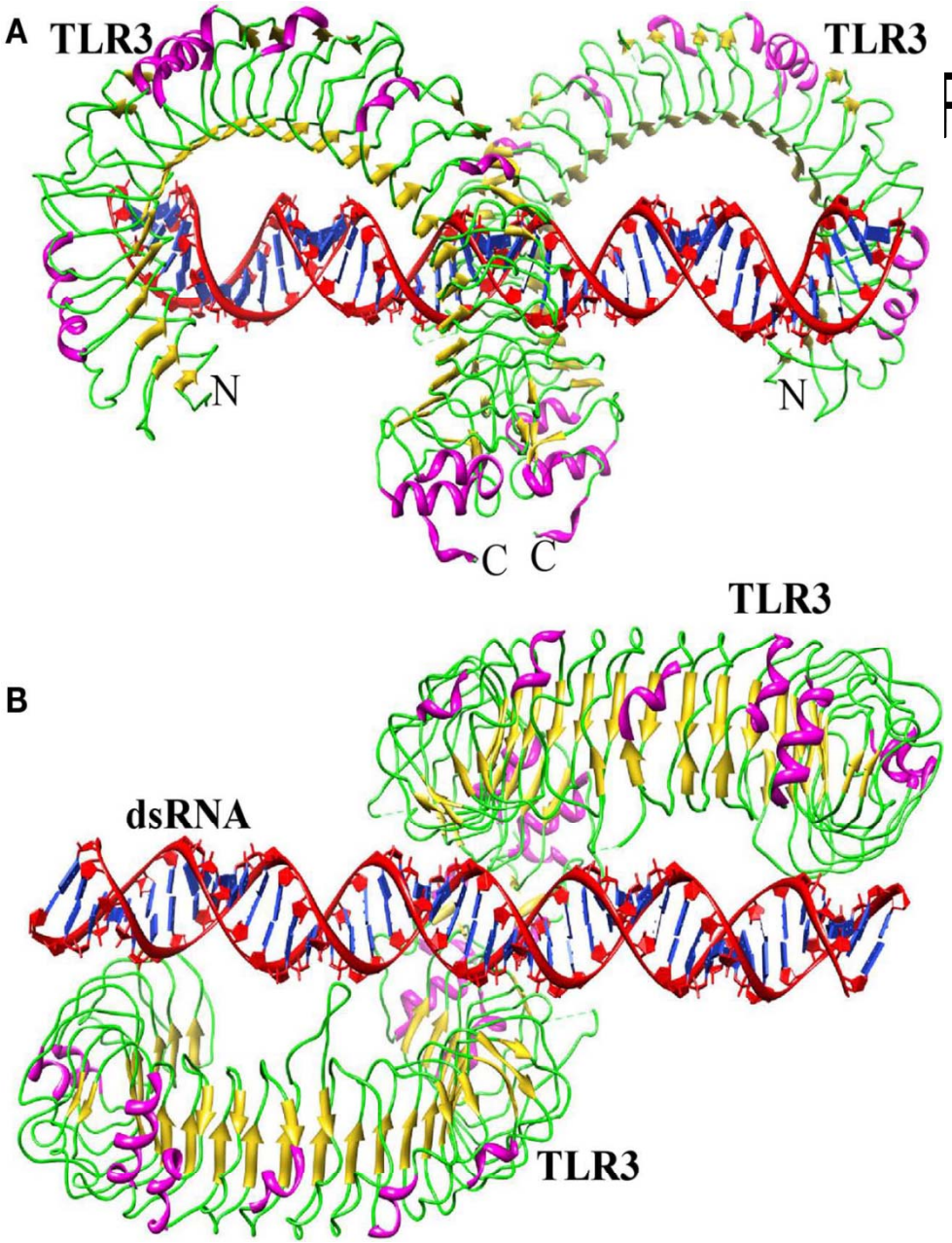
TIR

TLR:Ligand structures



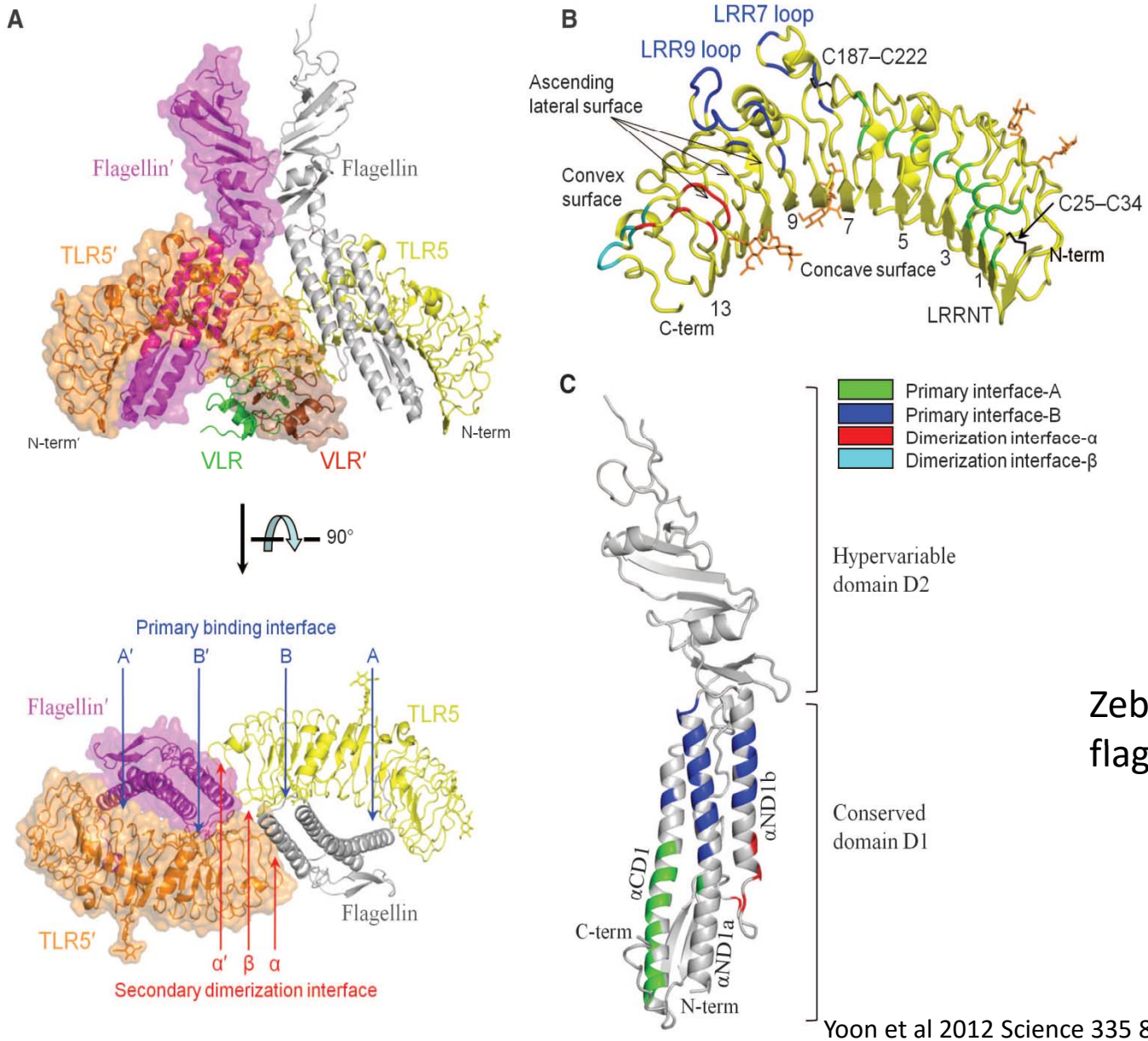
Manavalan et al 2011 Front Physiol 2(41) doi: 10.3389/fphys.2011.00041

R:Ligand structures



Manavalan et al 2011 Front Physiol 2(41) doi: 10.3389/fphys.2011.00041

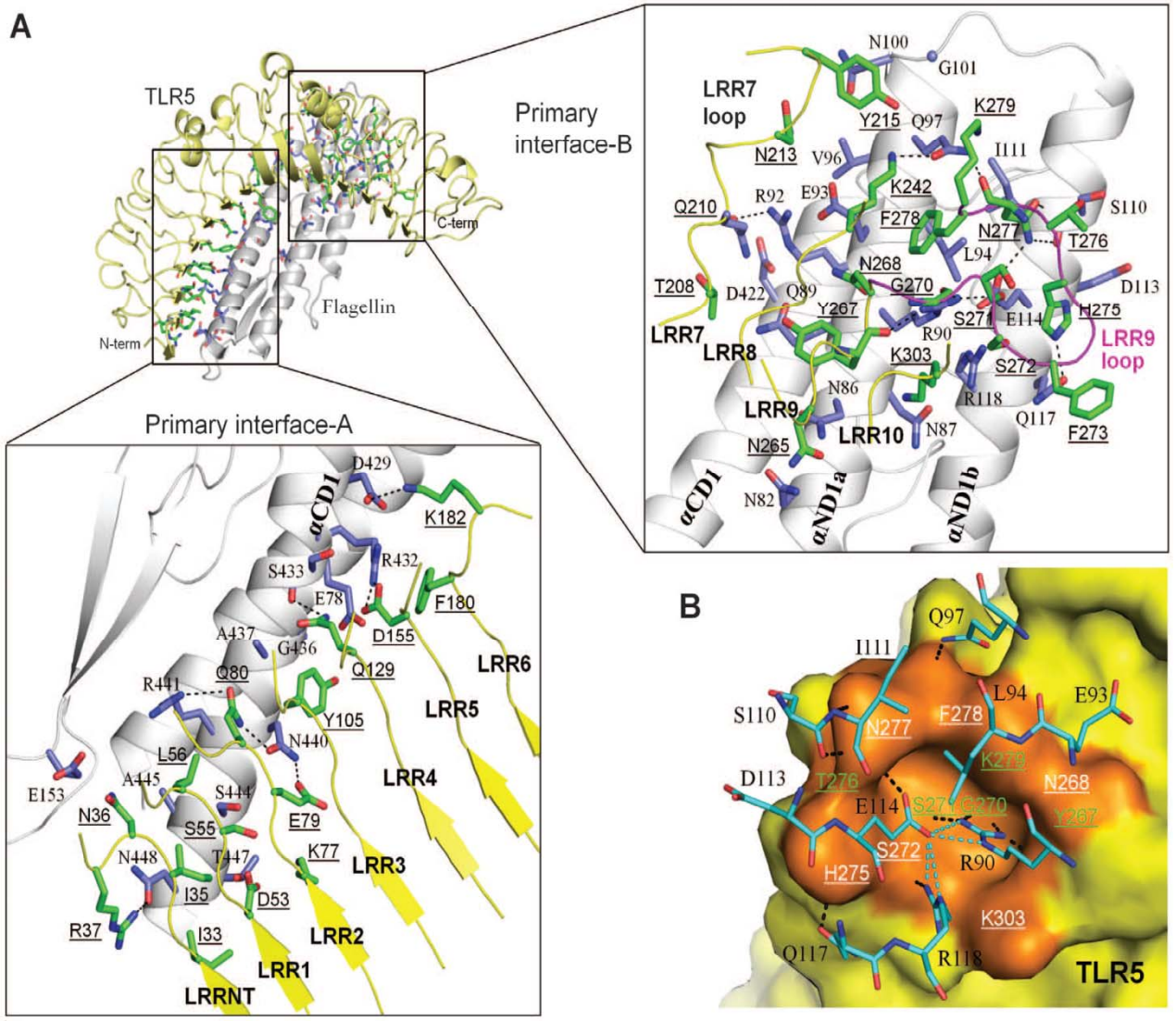
TLR:Ligand structures



Zebrafish TLR5 plus flagellin

Yoon et al 2012 Science 335 859-864 DOI: 10.1126/science.1215584

TLR:Ligand structures



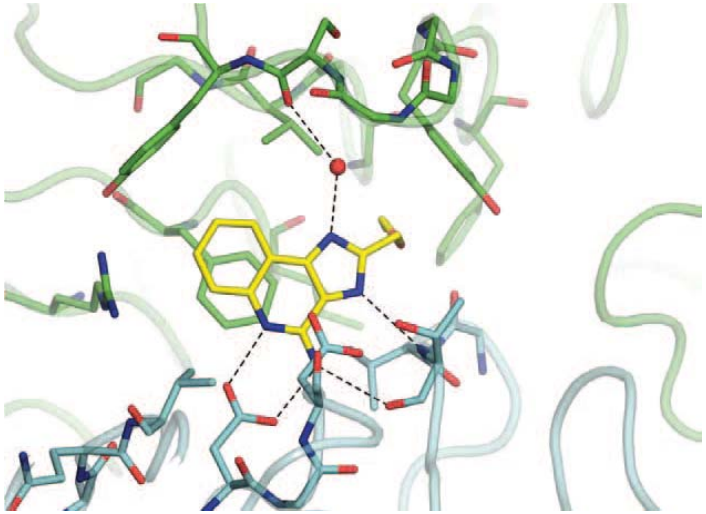
Zebrafish TLR5 plus flagellin

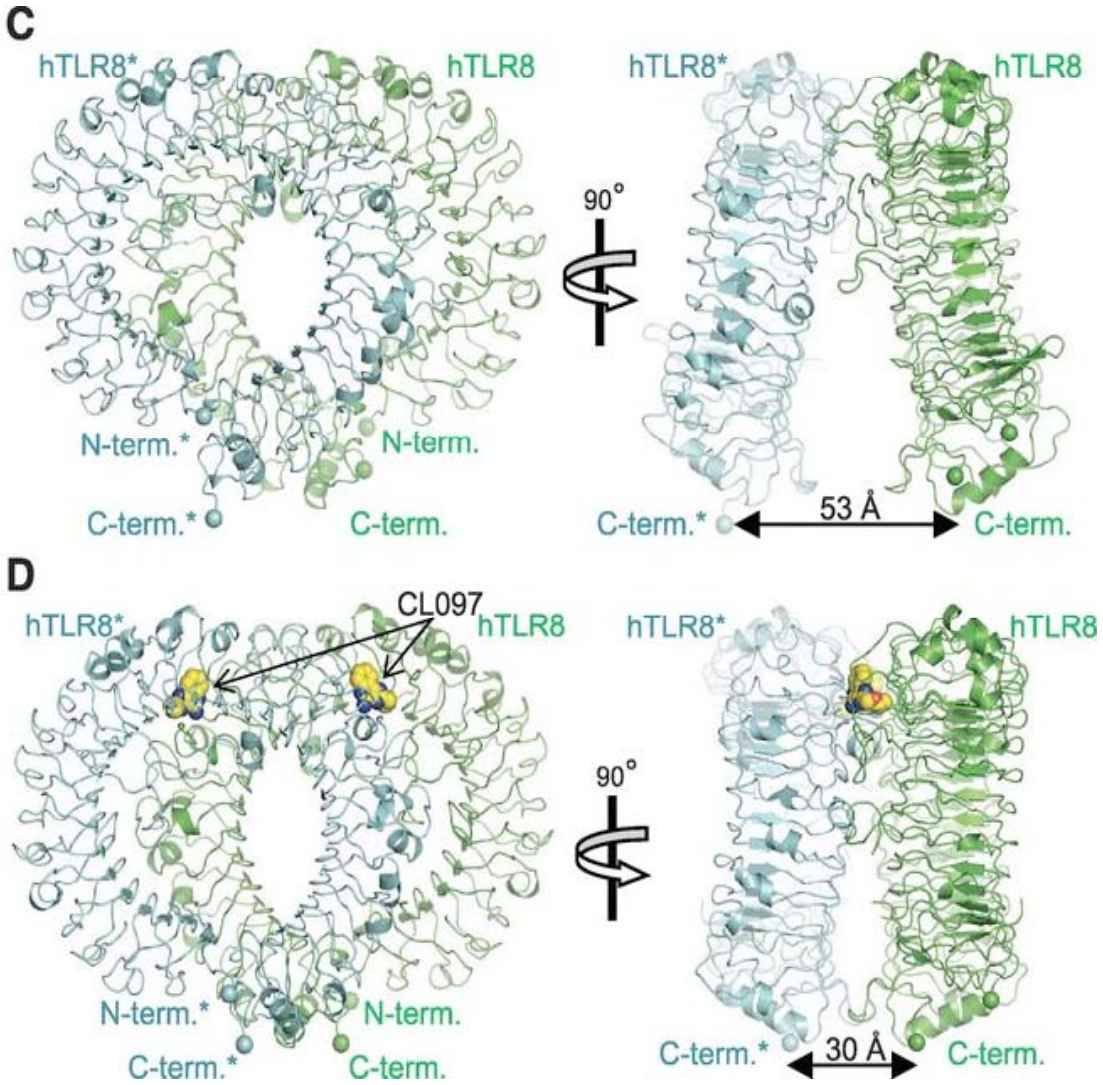
Yoon et al 2012 Science 335 859-864 DOI: 10.1126/science.1215584

TLR:Ligand structures

TLR8 bound to CL097, a thiazoloquinolone.

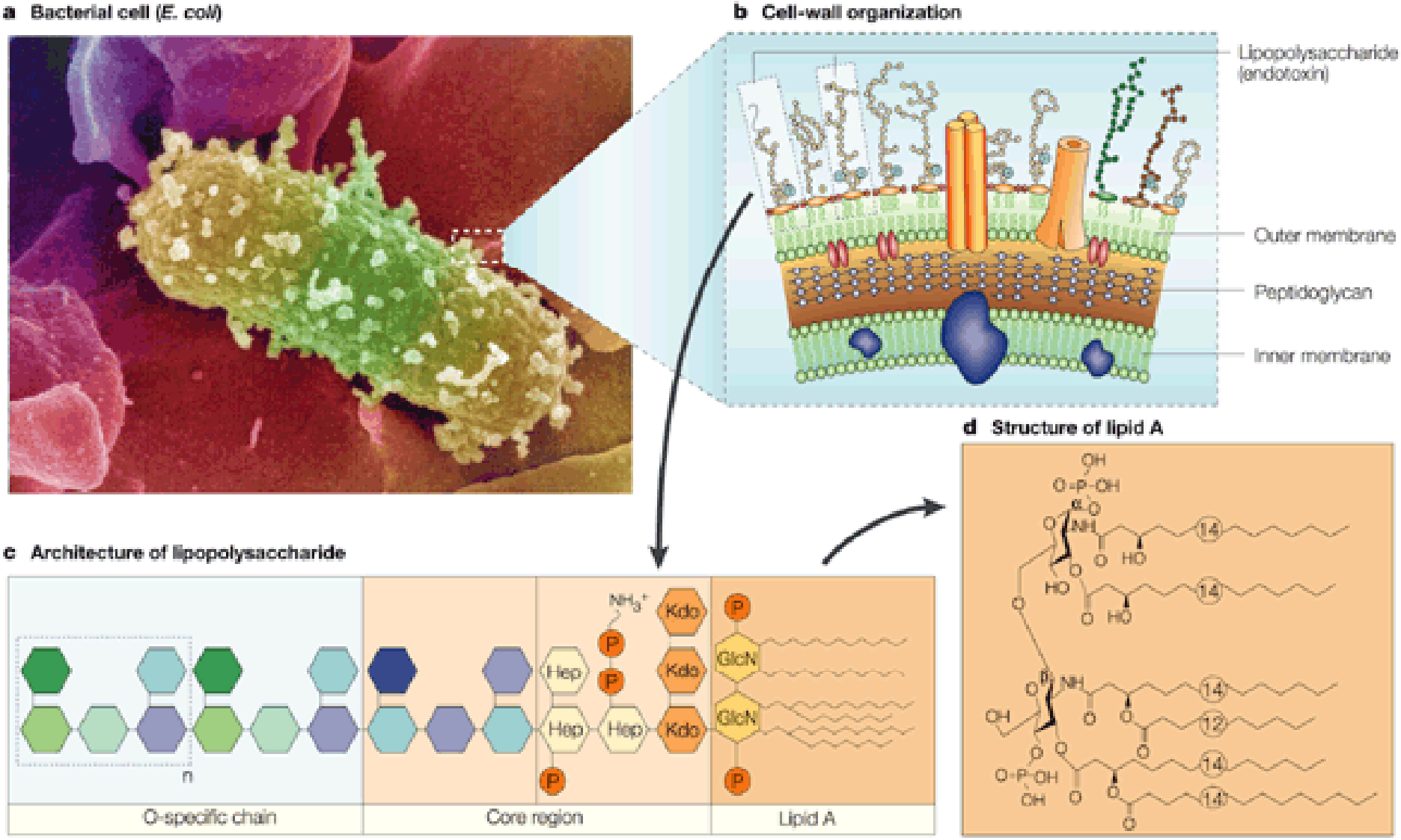
The aromatic ring of the ligand forms stacking interactions with Phe405.





Tanji et al. Science. 2013 Mar 22;339(6126):1426-9

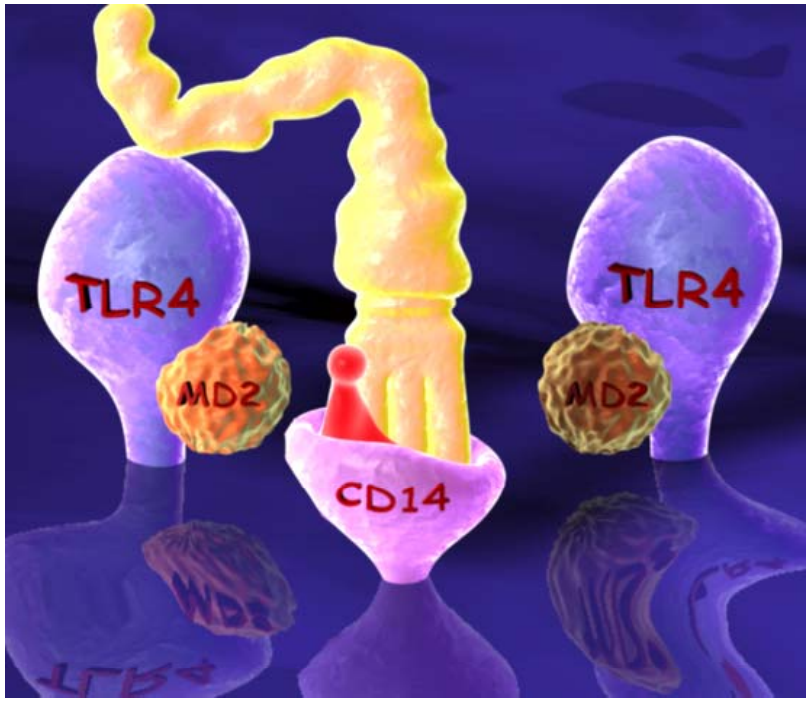
TLR4 as a model for PRR activation



Bruce Beutler

Nature Reviews | Immunology

Lipid A (biologically active component of LPS) recognition

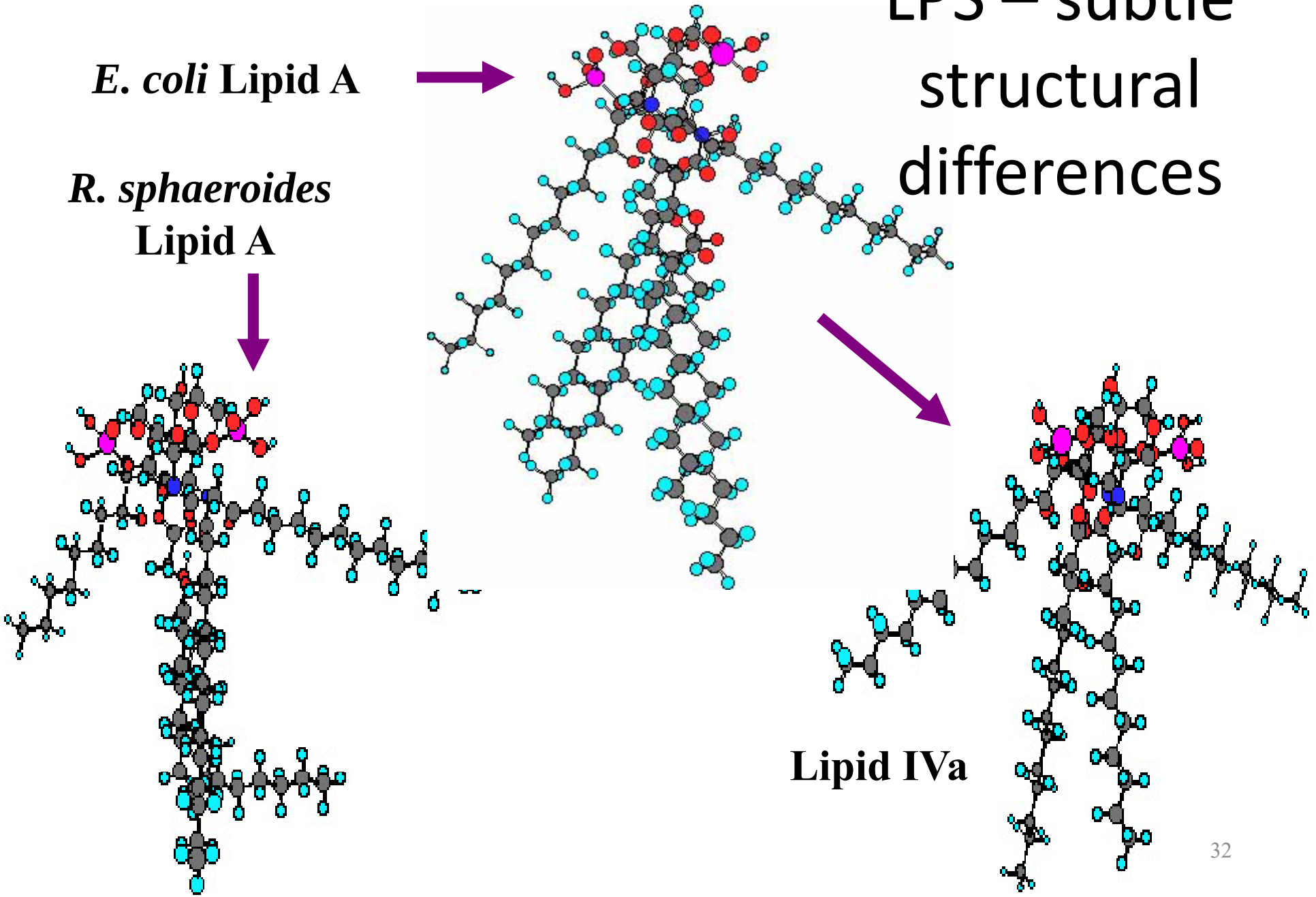


- LPS binds LPS binding protein
- LPS-LBP associates with CD-14
- CD-14 presents LPS to TLR-4-MD-2 complex
- TLR-4 dimerises and actives pro-inflammatory genes.

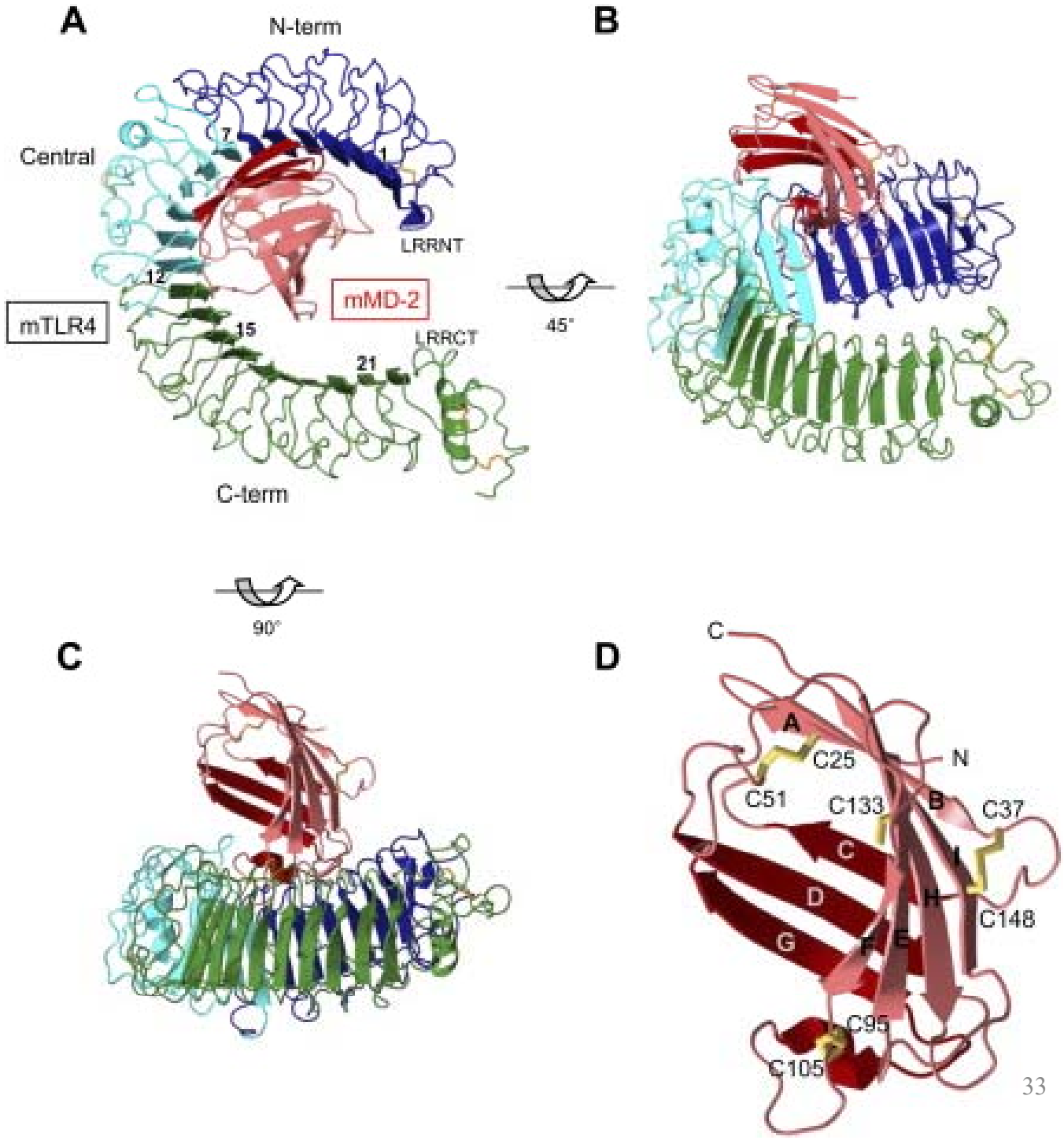
Ligand recognition by TLR-4

Ligand	Mouse	Human	Horse
E. coli LPS	+	+	+
S. typhimurium LPS	+	+	+
Taxol	+	0	+/0
Lipid IVa	+	-	PA
R. sphaeroides LPS	-	-	+
E5531	-	-	-
Penta-acylated LPS	+	0	?
Hexa-acylated LPS	+	+	?

LPS – subtle structural differences

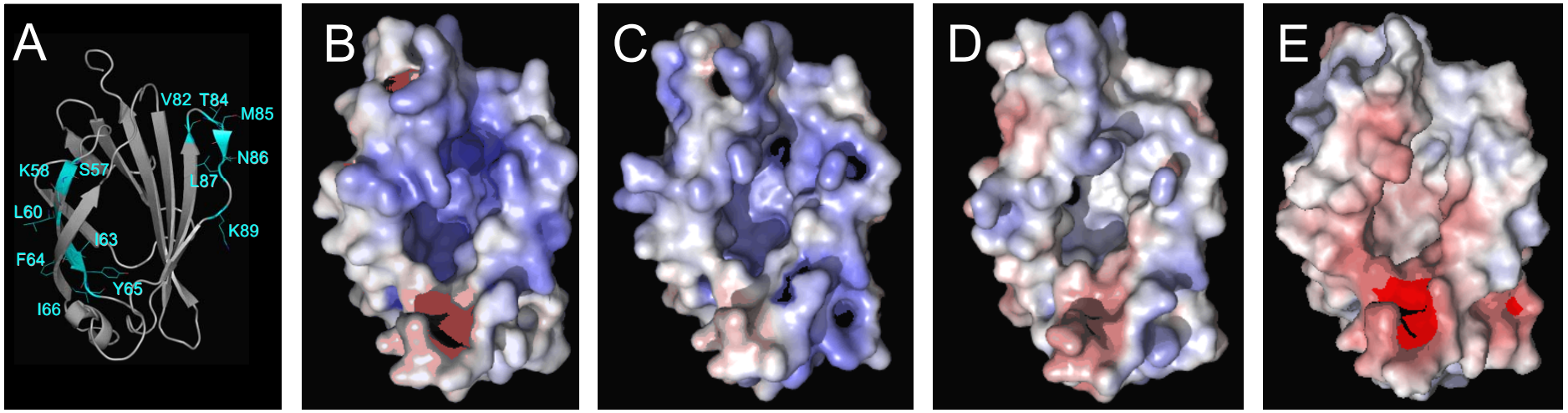


TLR4



See:
Kim et al Cell. 2007 Sep 7;130(5):906-17
And
Park et al Nature. 2009 30;458(7242):1191-5

MD-2 – crucial binding partner

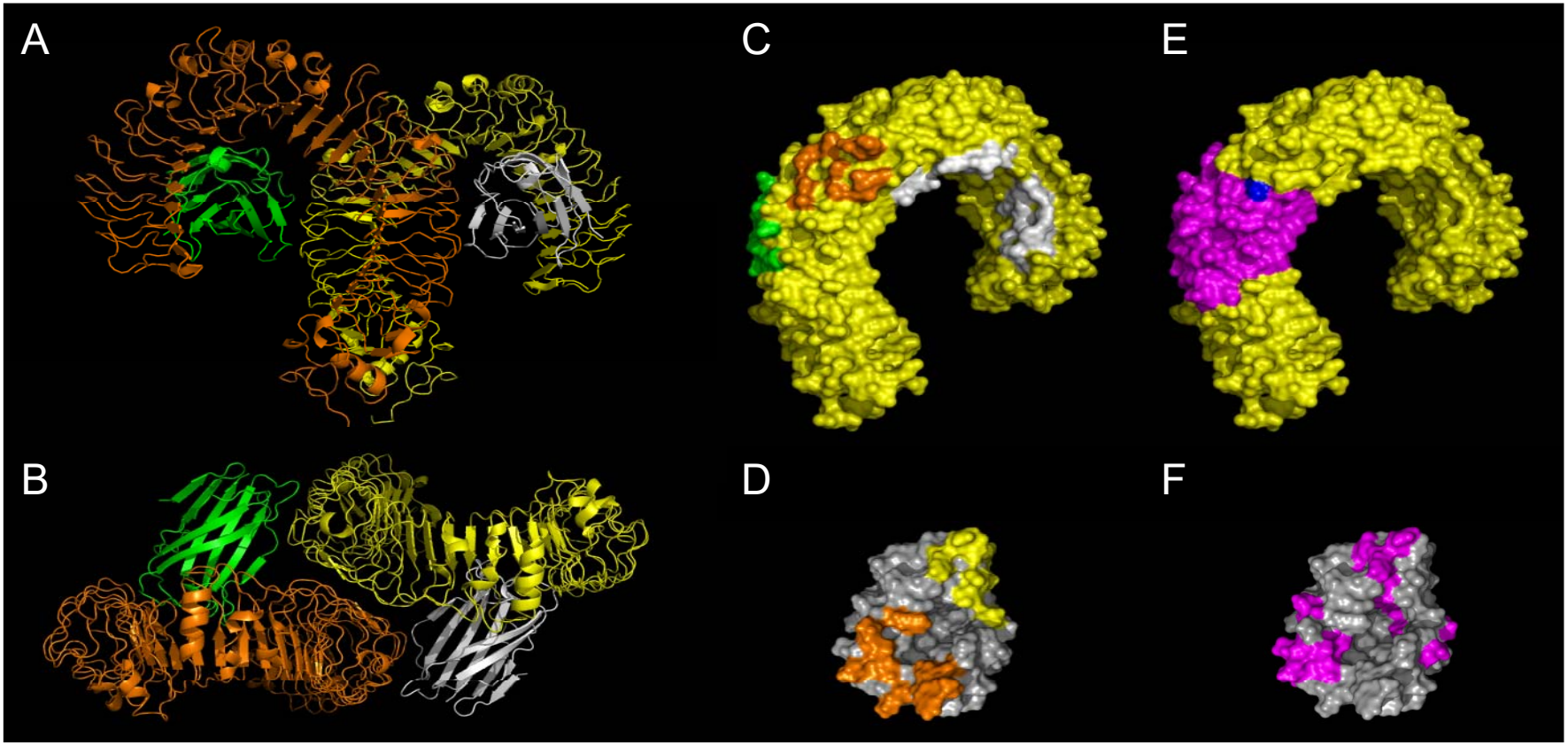


(A) Human MD-2 in ribbon representation: residues 57-66 and 82-89 (vary between human and horse) highlighted in cyan. Residues 57-66 are on beta-strand 4; residues 82-89 are on beta-strand 6a and surrounding loops lining the opening of ligand-binding cavity.
(B-E) Electrostatic surface potentials: human (B), cat (C), horse (D) and mouse (E) proteins.

Walsh et al. J Immunol. 2008 Jul 15;181(2):1245-54

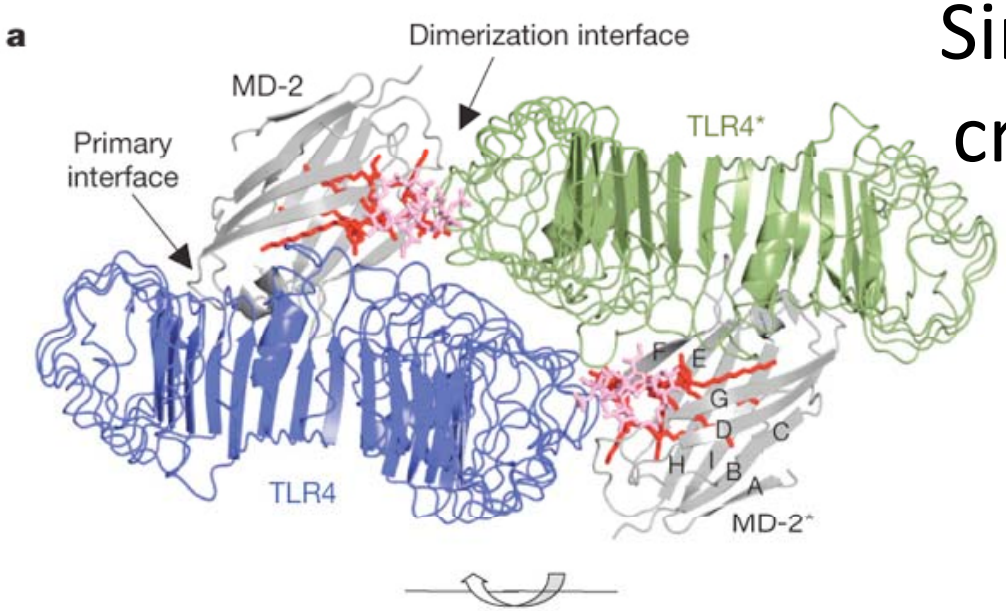
TLR4:MD2 interactions

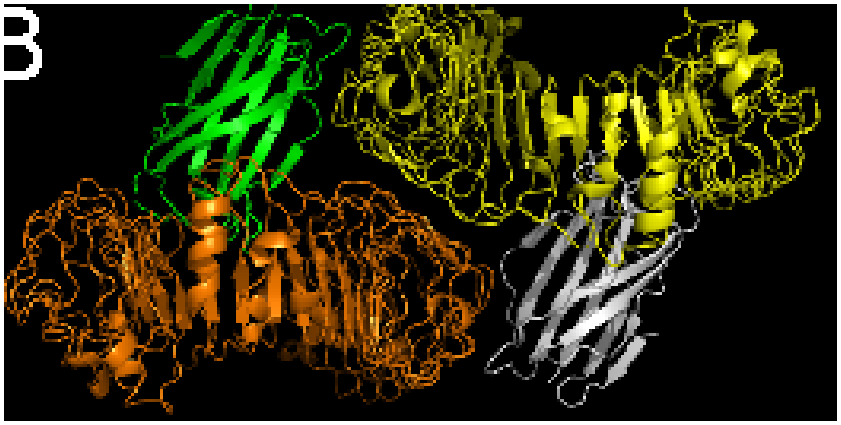
Models of TLR4:MD2 heterodimer (A, B). Surface of TLR4 showing important functional regions based on crystal structures and models (C,E) – white and green (MD2 binding), magenta (TLR4 dimerisation), Blue (equine R385). Surface of MD2 (D, F) with TLR4 binding sites (orange, yellow) overlapping residues 57-66 and 82-89, (magenta)

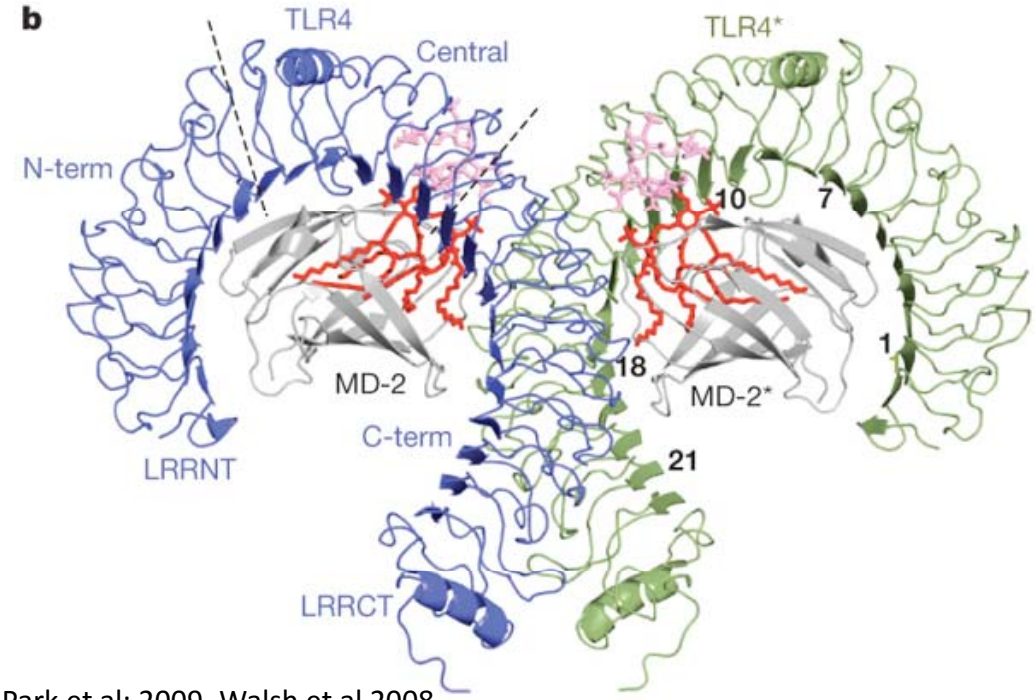


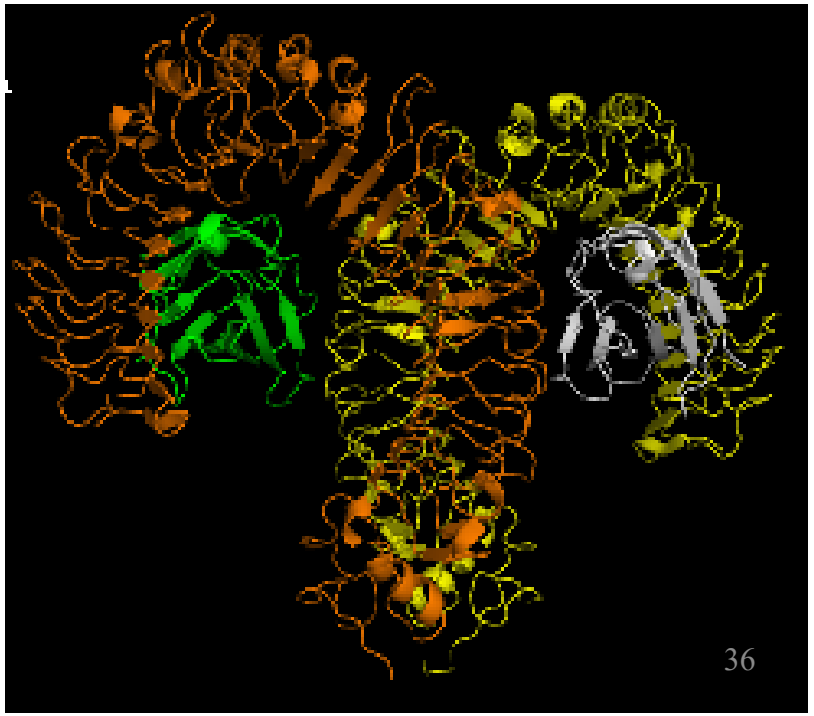
Walsh et al. J Immunol. 2008 Jul 15;181(2):1245-54

Similarity of model to crystallised structure



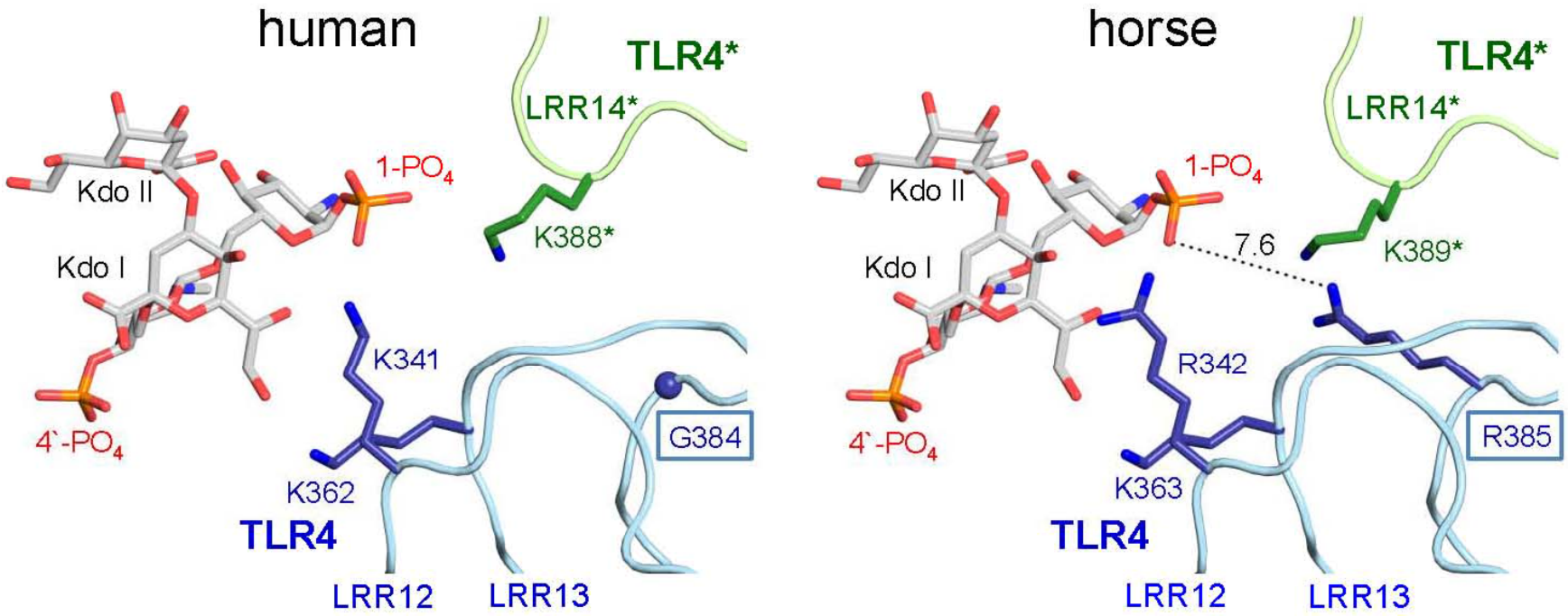






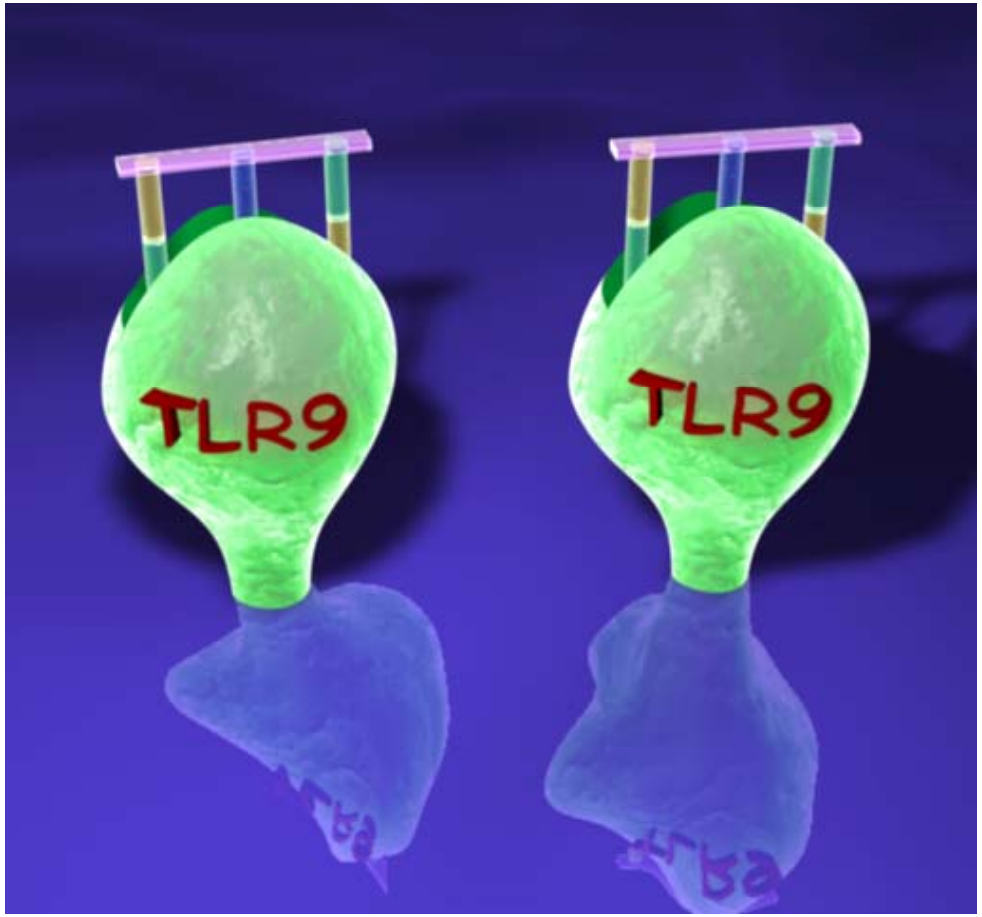
Park et al; 2009, Walsh et al 2008

Importance of R385



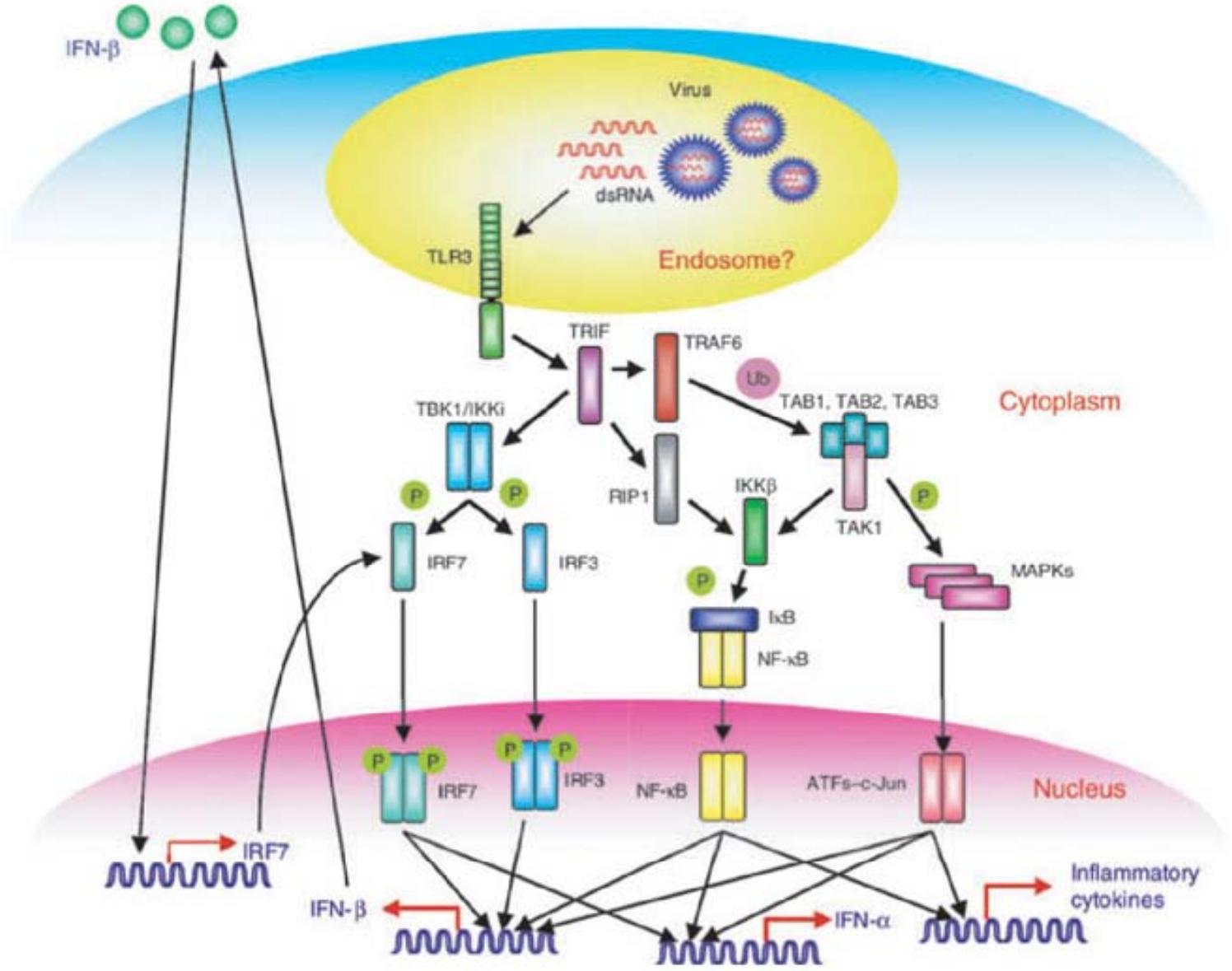
Park et al Nature. 2009 30;458(7242):1191-5

Other TLRs: RNA and DNA recognition by TLRs 3,7,8, 9



- eg lysis of bacteria by antibiotics releases methylated bacterial DNA
- Bacterial DNA binds to TLR-9
- TLR-9 dimerises and induces release of pro-inflammatory mediators

Viral recognition by TLRs: TLR3



Viral recognition by TLRs: 7,8,9